

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/44		A1	(11) International Publication Number: WO 98/13044
			(43) International Publication Date: 2 April 1998 (02.04.98)
(21) International Application Number: PCT/US97/14417		(72) Inventors: SLUSHER, Barbara, S.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). JACKSON, Paul, F.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). TAYS, Kevin, L.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US). MACLIN, Keith, M.; Guilford Pharmaceuticals Inc., 6611 Tributary Street, Baltimore, MD 21224 (US).	
(22) International Filing Date: 15 August 1997 (15.08.97)		(74) Agent: NATH, Gary, M.; Nath & Associates, 1835 K Street, N.W. #750, Washington, DC 20006-1203 (US).	
(30) Priority Data: 08/718,703 27 September 1996 (27.09.96) US 08/775,586 31 December 1996 (31.12.96) US 08/778,733 31 December 1996 (31.12.96) US 08/825,997 4 April 1997 (04.04.97) US 08/835,572 9 April 1997 (09.04.97) US 08/842,360 24 April 1997 (24.04.97) US 08/858,985 27 May 1997 (27.05.97) US 08/863,624 27 May 1997 (27.05.97) US 08/884,479 27 June 1997 (27.06.97) US		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant: GUILFORD PHARMACEUTICALS INC. [US/US]; 6611 Tributary Street, Baltimore, MD 21224 (US).		Published With international search report.	
(54) Title: PHARMACEUTICAL COMPOSITIONS AND METHODS OF TREATING COMPULSIVE DISORDERS USING NAALADASE INHIBITORS			
(57) Abstract			
The present invention relates to a pharmaceutical composition and a method for treating a compulsive disorder using a NAALADase inhibitor.			

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CJ	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

WO 98/13044

PCT/US97/14417

PHARMACEUTICAL COMPOSITIONS AND METHODS OF TREATING
COMPULSIVE DISORDERS USING NAALADASE INHIBITORS

This application is a continuation-in-part of U.S.
5 Patent Application No. 08/718,703, filed September 27,
1996, entitled "Treatment of Global and Focal Ischemia
Using NAALADase Inhibitors"; U.S. Patent Application No.
08/775,586, filed December 31, 1996, entitled "Inhibitors
of NAALADase Enzyme Activity"; U.S. Patent Application
10 No. 08/778,733, filed December 31, 1996, entitled
"Phosphoramidate Derivatives"; U.S. Patent Application
No. 08/825,997, filed April 4, 1997, entitled "Hydroxamic
Acid Derivatives"; U.S. Patent Application No.
08/835,572, filed April 9, 1997, entitled "Thio
15 Derivatives"; U.S. Patent Application No. 08/842,360,
filed April 24, 1997, entitled "Phosphonic Acid
Derivatives"; U.S. Patent Application filed May 27, 1997,
Attorney Docket No. 23029-X (serial number not yet
assigned), entitled "NAALADase Inhibitors"; U.S. Patent
20 Application filed May 27, 1997, Attorney Docket No.
23029-X2 (serial number not yet assigned), entitled
"NAALADase Inhibitors"; and U.S. Patent Application filed
June 27, 1997, Attorney Docket No. 23125-X (serial number
not yet assigned), entitled "Pharmaceutical Compositions
25 and Methods of Treating a Glutamate Abnormality and
Effecting a Neuronal Activity in an Animal Using
NAALADase Inhibitors", the entire contents of which

WO 98/13044

PCT/US97/14417

2

applications are herein incorporated by reference.

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

The present invention relates to a pharmaceutical composition and a method for treating a compulsive disorder using a NAALADase inhibitor.

10 2. Description of the Prior Art

Glutamate Abnormalities

Glutamate serves as the predominant excitatory neurotransmitter in the central nervous system (CNS). Neurons release glutamate in great quantities when they
15 are deprived of oxygen, as may occur during an ischemic brain insult such as a stroke or a heart attack. This excess release of glutamate in turn causes over-stimulation (excitotoxicity) of N-methyl-D-aspartate (NMDA), AMPA, Kainate and MGR receptors. When glutamate
20 binds to these receptors, ion channels in the receptors open, permitting flows of ions across their cell membranes, e.g., Ca^{2+} and Na^+ into the cells and K^+ out of the cells. These flows of ions, especially the influx of Ca^{2+} , cause over-stimulation of the neurons. The over-
25 stimulated neurons secrete more glutamate, creating a domino-effect which ultimately results in cell death via

WO 98/13044

PCT/US97/14417

3

the production of proteases, lipases and free radicals.

Excessive activation of glutamate receptors has been implicated in various neurological diseases and conditions, including epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, schizophrenia, chronic pain, ischemia and neuronal loss following hypoxia, hypoglycemia, ischemia, trauma, and nervous insult. Recent studies have also advanced a glutamatergic basis for compulsive disorders, particularly drug dependence.

As an example, neurophysiological and pathological effects of ethanol have been found to be mediated through the glutamatergic system. Specifically, acute exposure to ethanol disrupts glutamatergic neurotransmission by inhibiting ion flow through channels in glutamate receptors, whereas chronic exposure up-regulates the number of glutamate receptors and thereby increases ion flow. Acute withdrawal from ethanol results in hyperexcitability and seizures in the presence of up-regulated channels, thereby making postsynaptic neurons vulnerable to excitotoxic damage.

Post mortem examinations of histologically normal brains from alcoholics have shown that chronic alcoholism moderately increases the density of the NMDA subtype of glutamate receptors in the frontal cortex. This up-

WO 98/13044

PCT/US97/14417

4

regulation may represent a stage of ethanol-induced chronic neurotoxicity. As such, neurobiological effects of alcoholism, including intoxication, withdrawal seizures, delirium tremens, Wernicke-Korsakoff syndrome and fetal alcohol syndrome, can be understood as a spectrum of the consequences of ethanol's effect on the glutamatergic system. In this regard, alcoholism may be considered another member of the expanding family of glutamate-related neurological disorders.

10 The glutamatergic system has also been implicated in the behavioral effects of other abused drugs. For example, studies have shown that glutamatergic antagonists block motor-stimulating activities induced by amphetamine and cocaine, and glutamatergic agonists cause 15 the same stereotypy as that produced by amphetamine. These results represent pharmacological evidence that the expression of the stereotypic effect of psychomotor stimulants involves the glutamatergic system.

Epidemiologic studies have revealed a strong 20 correlation between drug dependence and other compulsive disorders. Additionally, a common genetic anomaly has been found among people with alcoholism, cocaine dependence, nicotine dependence, pathological gambling, attention deficit disorder (ADD), Tourette's syndrome, 25 compulsive overeating and obesity. Such disorders are believed to be manifestations of the effects of excitotoxicity.

WO 98/13044

PCT/US97/14417

5

Attempts to prevent excitotoxicity by blocking NMDA, AMPA, Kainate and MGR receptors have proven difficult because each receptor has multiple sites to which glutamate may bind. Many of the compositions that are effective in blocking the receptors are also toxic to animals. As such, there is currently no known effective treatment for glutamate abnormalities.

NAALADase Inhibitors

10 NAAG and NAALADase have been implicated in several human and animal pathological conditions. For example, it has been demonstrated that intra-hippocampal injections of NAAG elicit prolonged seizure activity. More recently, it was reported that rats genetically prone to epileptic seizures have a persistent increase in their basal level of NAALADase activity. These observations support the hypothesis that increased availability of synaptic glutamate elevates seizure susceptibility, and suggest that NAALADase inhibitors may provide anti-epileptic activity.

15 NAAG and NAALADase have also been implicated in the pathogenesis of ALS and in the pathologically similar animal disease called Hereditary Canine Spinal Muscular Atrophy (HCSMA). It has been shown that concentrations of NAAG and its metabolites -- NAA, glutamate and aspartate -- are elevated two- to three-fold in the

WO 98/13044

PCT/US97/14417

6

cerebrospinal fluid of ALS patients and HCSMA dogs. Additionally, NAALADase activity is significantly increased (two- to three-fold) in post-mortem spinal cord tissue from ALS patients and HCSMA dogs. As such, NAALADase inhibitors may be clinically useful in curbing the progression of ALS if increased metabolism of NAAG is responsible for the alterations of CSF levels of these acidic amino acids and peptides.

Abnormalities in NAAG levels and NAALADase activity have also been documented in post-mortem schizophrenic brain, specifically in the prefrontal and limbic brain regions.

The findings described above suggest that NAALADase inhibitors could be useful in treating glutamate abnormalities. In fact, the results of studies conducted by the inventors confirm that NAALADase inhibitors are effective in treating glutamate abnormalities, particularly stroke, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), spinal cord injury, alcoholism and nicotine dependence.

While a few NAALADase inhibitors have been identified, they have only been used in non-clinical research. Examples of such inhibitors include general metallopeptidase inhibitors such as o-phenanthroline, metal chelators such as EGTA and EDTA, and peptide analogs such as quisqualic acid and β -NAAG. Accordingly,

WO 98/13044

PCT/US97/14417

7

a need exists for new NAALADase inhibitors, as well as pharmaceutical compositions and methods using such new and known NAALADase inhibitors to treat glutamate abnormalities.

5

SUMMARY OF THE INVENTION

The present invention relates to a pharmaceutical composition comprising:

- (i) an effective amount of a NAALADase inhibitor
- 10 for treating a compulsive disorder; and
- (ii) a pharmaceutically acceptable carrier.

The present invention further relates to a method of treating a compulsive disorder, comprising administering an effective amount of a NAALADase inhibitor to a patient

15 in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph plotting in vitro toxicity of ischemic insult (potassium cyanide and 2-deoxyglucose)

20 against various doses of 2-(phosphonomethyl)pentanedioic acid with which cortical cell cultures were treated.

FIG. 2 is a bar graph plotting in vitro toxicity against various doses of NAAG to which cortical cell cultures were exposed.

25 FIG. 3 is a bar graph plotting in vitro toxicity following treatment with 2-(phosphonomethyl)pentanedioic

WO 98/13044

PCT/US97/14417

8

acid, against various doses of NAAG to which cortical cell cultures were exposed.

FIG. 4 is a bar graph plotting *in vitro* toxicity of ischemic insult against various times at which cortical
5 cell cultures were treated with 2-(phosphonomethyl)-pentanedioic acid.

FIG. 5 is a bar graph plotting *in vivo* cortical injury volume against various doses of 2-(phosphonomethyl)pentanedioic acid with which rats were
10 treated after sustaining middle cerebral artery occlusion.

FIG. 6 is a bar graph plotting *in vivo* total brain infarct volume of rats against various times at which the rats are treated with 2-(phosphonomethyl)pentanedioic
15 acid after sustaining middle cerebral artery occlusion.

FIG. 7 is a bar graph plotting *in vivo* extracellular glutamate increases in the striatum of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

20 FIG. 8 is a bar graph plotting *in vivo* extracellular glutamate increases in the parietal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid after sustaining middle cerebral artery occlusion.

FIG. 9 is a bar graph plotting *in vivo* extracellular
25 glutamate increases in the frontal cortex of rats treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid

WO 98/13044

PCT/US97/14417

9

after sustaining middle cerebral artery occlusion.

FIG. 10(a) is a photomicrograph of mouse sciatic nerve treated with a vehicle following cryolesion.

FIG. 10(b) is a photomicrograph of mouse sciatic
5 nerve treated with 2-(phosphonomethyl)pentanedioic acid following cryolesion.

FIG. 11 is a bar graph plotting percent striatal TH innervation density against the treatment of mice with vehicle alone, vehicle following MPTP, or 2-
10 (phosphonomethyl)pentanedioic acid following MPTP.

FIG. 12 is a bar graph plotting the neurological function code against the treatment of rats with dynorphin A alone or 2-(phosphonomethyl)pentanedioic acid with dynorphin A.

15 FIG. 13 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against the treatment of the cultures with 2-(phosphonomethyl)-pentanedioic acid alone, threohydroxyaspartate (THA) alone, or THA with 2-(phosphonomethyl)pentanedioic acid.

20 FIG. 14 is a bar graph plotting the ChAT activity of rat spinal cord organotypic cultures against various doses of 2-(phosphonomethyl)pentanedioic acid with which the cultures were treated in the presence of THA.

FIG. 15 is a bar graph plotting the ethanol intake
25 of alcohol-preferring rats against various doses of 2-(phosphonomethyl)pentanedioic acid with which the rats

WO 98/13044

PCT/US97/14417

10

were treated.

FIG. 16 is a graph plotting the cumulative nicotine intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

FIG. 17 is a graph plotting the cumulative food intake of rats during a 1 hour test session, before which the rats had been trained to self-administer nicotine and pretreated with a vehicle or 2-(phosphonomethyl)-pentanedioic acid.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Attention Deficit Disorder" refers to a disorder characterized by developmentally inappropriate inattention and impulsivity, with or without hyperactivity. Inattention means a failure to finish tasks started, easy distractibility, seeming lack of attention, and difficulty concentrating on tasks requiring sustained attention. Impulsivity means acting before thinking, difficulty taking turns, problems organizing work, and constant shifting from one activity to another. Hyperactivity means difficulty staying seated and sitting still, and running or climbing excessively.

WO 98/13044

PCT/US97/14417

11

"Compound 3" refers to 2-(phosphonomethyl)pentane-dioic acid (PMPA).

"Compulsive disorder" refers to any disorder characterized by irresistible impulsive behavior.

5 Examples of compulsive disorders include without limitation drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

"Drug dependence" refers to a psychologic addiction or a physical tolerance to a drug. Tolerance means a
10 need to increase the dose progressively in order to produce the effect originally achieved by smaller amounts.

"Eating disorder" refers to compulsive overeating, obesity or severe obesity. Obesity means body weight of
15 20% over standard height-weight tables. Severe obesity means over 100% overweight.

"Glutamate abnormality" refers to any disease, disorder or condition in which glutamate is implicated, including pathological conditions involving elevated
20 levels of glutamate. Examples of glutamate abnormalities include epilepsy, stroke, Alzheimer's disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's Disease, schizophrenia, chronic pain, ischemia, neuronal insult and compulsive disorders.

25 "Glutamate modulator" refers to any composition of matter which alone or in combination with another agent

WO 98/13044

PCT/US97/14417

12

affects the level of glutamate in an animal.

"Inhibition", in the context of enzymes, refers to reversible enzyme inhibition such as competitive, uncompetitive and non-competitive inhibition.

5 Competitive, uncompetitive and non-competitive inhibition can be distinguished by the effects of an inhibitor on the reaction kinetics of an enzyme. Competitive inhibition occurs when the inhibitor combines reversibly with the enzyme in such a way that it competes with a
10 normal substrate for binding at the active site. The affinity between the inhibitor and the enzyme may be measured by the inhibitor constant, K_i , which is defined as:

15
$$K_i = \frac{[E][I]}{[EI]}$$

wherein [E] is the concentration of the enzyme, [I] is
20 the concentration of the inhibitor, and [EI] is the concentration of the enzyme-inhibitor complex formed by the reaction of the enzyme with the inhibitor. Unless otherwise specified, K_i as used herein refers to the affinity between the inventive compounds and NAALADase.
25 "IC₅₀" is a related term used to define the concentration or amount of a compound which is required to cause a 50% inhibition of the target enzyme.

WO 98/13044

PCT/US97/14417

13

"Ischemia" refers to localized tissue anemia due to obstruction of the inflow of arterial blood. Global ischemia occurs when blood flow to the entire brain ceases for a period of time, such as may result from cardiac arrest. Focal ischemia occurs when a portion of the brain is deprived of its normal blood supply, such as may result from thromboembolytic occlusion of a cerebral vessel, traumatic head injury, edema or brain tumor. Even if transient, both global and focal ischemia can produce widespread neuronal damage. Although nerve tissue damage occurs over hours or even days following the onset of ischemia, some permanent nerve tissue damage may develop in the initial minutes following cessation of blood flow to the brain. Much of this damage is attributed to glutamate toxicity and secondary consequences of reperfusion of the tissue, such as the release of vasoactive products by damaged endothelium, and the release of cytotoxic products, such as free radicals and leukotrienes, by the damaged tissue.

"NAAG" refers to N-acetyl-aspartyl-glutamate, an important peptide component of the brain, with levels comparable to the major inhibitor neurotransmitter gamma-aminobutyric acid (GABA). NAAG is neuron-specific, present in synaptic vesicles and released upon neuronal stimulation in several systems presumed to be glutamatergic. Studies suggest that NAAG may function as

WO 98/13044

PCT/US97/14417

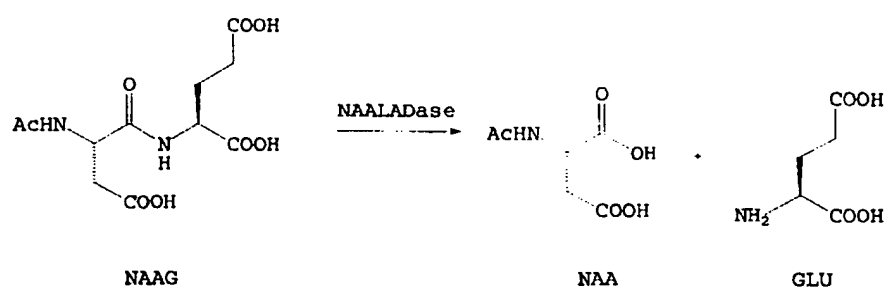
14

a neurotransmitter and/or neuromodulator in the central nervous system, or as a precursor of the neurotransmitter glutamate.

"NAALADase" refers to N-acetylated α -linked acidic dipeptidase, a membrane-bound metallopeptidase which catabolizes NAAG to N-acetylaspartate (NAA) and glutamate:

Catabolism of NAAG by NAALADase

10



NAALADase shows a high affinity for NAAG with a K_m of 540 nM. If NAAG is a bioactive peptide, then NAALADase may serve to inactivate NAAG'S synaptic action. Alternatively, if NAAG functions as a precursor for glutamate, the primary function of NAALADase may be to regulate synaptic glutamate availability.

"Nervous function" refers to the various functions of the nervous system, which among other things provide an awareness of the internal and external environments of

WO 98/13044

PCT/US97/14417

15

the body, make possible voluntary and reflex activities between the various structural elements of the organism, and balance the organism's response to environmental changes.

5 "Nervous insult" refers to any damage to nervous tissue and any disability or death resulting therefrom. The cause of nervous insult may be metabolic, toxic, neurotoxic, iatrogenic, thermal or chemical, and includes without limitation ischemia, hypoxia, cerebrovascular
10 accident, trauma, surgery, pressure, mass effect, hemorrhage, radiation, vasospasm, neurodegenerative disease, neurodegenerative process, infection, Parkinson's disease, ALS, myelination/demyelination process, epilepsy, cognitive disorder, glutamate
15 abnormality and secondary effects thereof. Currently, there is no known effective treatment for nervous tissue damage.

"Nervous tissue" refers to the various components that make up the nervous system, including without
20 limitation neurons, neural support cells, glia, Schwann cells, vasculature contained within and supplying these structures, the central nervous system, the brain, the brain stem, the spinal cord, the junction of the central nervous system with the peripheral nervous system, the
25 peripheral nervous system and allied structures.

WO 98/13044

PCT/US97/14417

16

"Neuroprotective" refers to the effect of reducing, arresting or ameliorating nervous insult, and protecting, resuscitating or reviving nervous tissue which has suffered nervous insult.

5 "Pathological gambling" is a condition characterized by a preoccupation with gambling. Similar to psychoactive substance abuse, its effects include development of tolerance with a need to gamble progressively larger amounts of money, withdrawal
10 symptoms, and continued gambling despite severe negative effects on family and occupation.

"Pharmaceutically acceptable salt" refers to a salt of the inventive compounds which possesses the desired pharmacological activity and which is neither
15 biologically nor otherwise undesirable. The salt can be formed with inorganic acids such as acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate,
20 dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate heptanoate, hexanoate, hydrochloride hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate,
25 oxalate, thiocyanate, tosylate and undecanoate. Examples of a base salt include ammonium salts, alkali metal salts

WO 98/13044

PCT/US97/14417

17

such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine and lysine. The basic nitrogen-containing groups can be quarternized with agents including lower alkyl halides such as methyl, ethyl, propyl and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aralkyl halides such as benzyl and phenethyl bromides.

"Tourette's syndrome" refers to an autosomal multiple tic disorder characterized by compulsive swearing, multiple muscle tics and loud noises. Tics are brief, rapid, involuntary movements that can be simple or complex; they are stereotyped and repetitive, but not rhythmic. Simple tics, such as eye blinking, often begin as nervous mannerisms. Complex tics often resemble fragments of normal behavior.

"Treating" refers to:

(i) preventing a disease, disorder or condition from occurring in an animal which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

WO 98/13044

PCT/US97/14417

18

(ii) inhibiting the disease, disorder or condition,
i.e., arresting its development; and

(iii) relieving the disease, disorder or condition,
i.e., causing regression of the disease, disorder and/or
5 condition.

In relation to drug dependence, "treating" refers to
suppressing the psychologic addiction or physical
tolerance to the drug of abuse, and relieving or
preventing a withdrawal syndrome resulting from the drug
10 dependence.

"Withdrawal syndrome" refers to a disorder
characterized by untoward physical changes that occur
when the drug is discontinued or when its effect is
counteracted by a specific antagonist.

15

PHARMACEUTICAL COMPOSITIONS OF THE PRESENT INVENTION

The present invention relates to a pharmaceutical
composition comprising:

- (i) an effective amount of a NAALADase inhibitor
20 for treating a compulsive disorder; and
(ii) a pharmaceutically acceptable carrier.

The pharmaceutical composition may further comprise
at least one additional therapeutic agent.

Since NAALADase is a metallopeptidase, useful
25 NAALADase inhibitors for the pharmaceutical composition
of the present invention include small molecule compounds

WO 98/13044

PCT/US97/14417

19

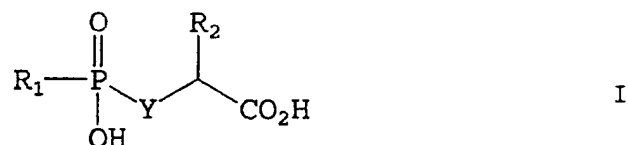
with functional groups known to inhibit metallo-peptidases, such as hydroxyphosphinyl derivatives.

According to scientific literature, the glutamate moiety plays a more critical role than the aspartate moiety in the recognition of NAAG by NAALADase. As such, a preferred NAALADase inhibitor is a glutamate-derived hydroxyphosphinyl derivative, an acidic peptide analog, a conformationally restricted glutamate mimic or a mixture thereof.

10 A preferred acidic peptide analog is selected from the group consisting of Asp-Glu, Glu-Glu, Gly-Glu, gamma-Glu-Glu and Glu-Glu-Glu.

A preferred NAALADase inhibitor is a glutamate-derived hydroxyphosphinyl derivative of formula I:

15



20 or a pharmaceutically acceptable salt or hydrate thereof, wherein:

Y is CR₃R₄, NR₅ or O;

R₁ and R₅ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is

25

WO 98/13044

PCT/US97/14417

20

unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-
5 C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₂ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
10 C₇ cycloalkenyl and Ar, wherein said R₂ is unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy,
15 phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₃ and R₄ are independently selected from the group consisting of hydrogen, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, Ar, halo and mixtures
20 thereof;

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl,
25 benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl,

WO 98/13044

PCT/US97/14417

21

C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

Preferably, Y is CH₂.

5 More preferably, R₂ is substituted with carboxy.

Even more preferably, R₁ is hydrogen, C₁-C₄ straight or branched chain alkyl, C₂-C₄ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, benzyl or phenyl, wherein said R₁ is unsubstituted or substituted
10 with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, benzyl, phenyl or mixtures thereof; and
15 R₂ is C₁-C₂ alkyl.

Most preferably, the glutamate-derived hydroxyphosphinyl derivative is selected from the group consisting of:

2- (phosphonomethyl)pentanedioic acid;

20 2- (phosphonomethyl)succinic acid;

2- [[[2-carboxyethyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2- [[methylhydroxyphosphinyl)methyl]pentanedioic acid;

2- [[ethylhydroxyphosphinyl)methyl]pentanedioic acid;

25 2- [[propylhydroxyphosphinyl)methyl]pentanedioic acid;

2- [[butylhydroxyphosphinyl)methyl]pentanedioic acid;

WO 98/13044

PCT/US97/14417

22

- 2- [[cyclohexylhydroxyphosphinyl]methyl]pentanedioic acid;
2- [[(cyclohexyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2- [[phenylhydroxyphosphinyl]methyl]pentanedioic acid;
5 2- [(benzylhydroxyphosphinyl)methyl]pentanedioic acid;
2- [[(phenylmethyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2- [[(phenylethyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
10 2- [[(phenylpropyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2- [[(phenylbutyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2- [[(4-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic
15 acid;
2- [[(4-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2- [[(2-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
20 2- [[(pentafluorobenzyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
2- [[(methoxybenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2- [[(2,3,4-trimethoxyphenyl)hydroxyphosphinyl]methyl] -
25 pentanedioic acid;
2- [[(phenylprop-2-enyl)hydroxyphosphinyl]methyl]pentane-

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

23

dioic acid;

2-[[[(2-fluorobenzyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(hydroxy)phenylmethyl)hydroxyphosphinyl)methyl]-
5 pentanedioic acid;

2-[[[(3-methylbenzyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(4-fluorophenyl)hydroxyphosphinyl)methyl]pentanedioic acid;

10 2-[[[(3-trifluoromethylbenzyl)hydroxyphosphinyl)methyl]-
pentanedioic acid; and

pharmaceutically acceptable salts and hydrates thereof.

In other embodiments, R_2 is C_3 - C_9 alkyl; R_1 is 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl,
15 tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl or C_1 - C_4 straight or branched chain alkyl substituted with 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-
20 pyridyl or 4-pyridyl; or R_1 is 1-naphthyl, 2-naphthyl, or C_1 - C_4 straight or branched chain alkyl substituted with 1-naphthyl or 2-naphthyl.

Preferred compounds of these embodiments include:

2-[(methylhydroxyphosphinyl)methyl]hexanedioic acid;

25 2-[(benzylhydroxyphosphinyl)methyl]hexanedioic acid;

2-[(methylhydroxyphosphinyl)methyl]heptanedioic acid;

WO 98/13044

PCT/US97/14417

24

- 2-[(benzylhydroxyphosphinyl)methyl]heptanedioic acid;
2-[(methylhydroxyphosphinyl)methyl]octanedioic acid;
2-[(benzylhydroxyphosphinyl)methyl]octanedioic acid;
2-[(methylhydroxyphosphinyl)methyl]nonanedioic acid;
5 2-[(benzylhydroxyphosphinyl)methyl]nonanedioic acid;
2-[(methylhydroxyphosphinyl)methyl]decanedioic acid;
2-[(benzylhydroxyphosphinyl)methyl]decanedioic acid;
2-[[(2-pyridyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
10 2-[[(3-pyridyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(4-pyridyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(3-pyridyl)ethylhydroxyphosphinyl]methyl]pentane-
15 dioic acid;
2-[[(3-pyridyl)propylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(tetrahydrofuranyl)methylhydroxyphosphinyl]methyl]-
pentanedioic acid;
20 2-[[(tetrahydrofuranyl)ethylhydroxyphosphinyl]methyl]-
pentanedioic acid;
2-[[(tetrahydrofuranyl)propylhydroxyphosphinyl]methyl]-
pentanedioic acid;
2-[[(2-tetrahydropyranyl)hydroxyphosphinyl]methyl]-
25 pentanedioic acid;
2-[[(3-tetrahydropyranyl)hydroxyphosphinyl]methyl]-

WO 98/13044

PCT/US97/14417

25

- pentanedioic acid;
- 2-[[(4-tetrahydropyranyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;
- 2-[[(2-indolyl)methylhydroxyphosphinyl]methyl]pentane-
5 dioic acid;
- 2-[[(3-indolyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(4-indolyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 10 2-[[(3-indolyl)ethylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(3-indolyl)propylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(2-thienyl)methylhydroxyphosphinyl]methyl]pentane-
15 dioic acid;
- 2-[[(3-thienyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(4-thienyl)methylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 20 2-[[(3-thienyl)ethylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(3-thienyl)propylhydroxyphosphinyl]methyl]pentane-
dioic acid;
- 2-[[(2-pyridyl)hydroxyphosphinyl]methyl]pentanedioic
25 acid;
- 2-[[(3-pyridyl)hydroxyphosphinyl]methyl]pentanedioic

WO 98/13044

PCT/US97/14417

26

acid;

2-[[[(4-pyridyl)hydroxyphosphinyl)methyl]pentanedioic
acid;5 2-[[[(tetrahydrofuranyl)hydroxyphosphinyl)methyl]pentane-
dioic acid;2-[[[(2-indolyl)hydroxyphosphinyl)methyl]pentanedioic
acid;2-[[[(3-indolyl)hydroxyphosphinyl)methyl]pentanedioic
acid;10 2-[[[(4-indolyl)hydroxyphosphinyl)methyl]pentanedioic
acid;2-[[[(2-thienyl)hydroxyphosphinyl)methyl]pentanedioic
acid;15 2-[[[(3-thienyl)hydroxyphosphinyl)methyl]pentanedioic
acid;2-[[[(4-thienyl)hydroxyphosphinyl)methyl]pentanedioic
acid;2-[[[(1-naphthyl)hydroxyphosphinyl)methyl]pentanedioic
acid;20 2-[[[(2-naphthyl)hydroxyphosphinyl)methyl]pentanedioic
acid;2-[[[(1-naphthyl)methylhydroxyphosphinyl)methyl]pentane-
dioic acid;25 2-[[[(2-naphthyl)methylhydroxyphosphinyl)methyl]pentane-
dioic acid;

2-[[[(1-naphthyl)ethylhydroxyphosphinyl)methyl]pentane-

WO 98/13044

PCT/US97/14417

27

dioic acid;
2-[[[(2-naphthyl)ethylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[[(1-naphthyl)propylhydroxyphosphinyl]methyl]pentane-
5 dioic acid;
2-[[[(2-naphthyl)propylhydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[[(1-naphthyl)butylhydroxyphosphinyl]methyl]pentane-
dioic acid;
10 2-[[[(2-naphthyl)butylhydroxyphosphinyl]methyl]pentane-
dioic acid; and
pharmaceutically acceptable salts and hydrates thereof.

In another preferred embodiment, Y is CH₂ and R₂ is selected from the group consisting of hydrogen, C₁-C₉,
15 straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, benzyl and phenyl, wherein said R₂ is unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, C₁-C₆ straight or branched chain alkyl, C₂-
20 C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, phenyl or mixtures thereof.

More preferably, R₁ is hydrogen, C₁-C₄ straight or branched chain alkyl, C₂-C₄ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, benzyl or
25 phenyl, wherein said R₁ is unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo,

WO 98/13044

PCT/US97/14417

28

hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, benzyl, phenyl or mixtures thereof.

5 Most preferably, the glutamate-derived hydroxyphosphinyl derivative is selected from the group consisting of:

3-(methylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(ethylhydroxyphosphinyl)-2-phenylpropanoic acid;

10 3-(propylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(butylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(cyclohexylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-((cyclohexyl)methylhydroxyphosphinyl)-2-phenylpropanoic acid;

15 3-(phenylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(phenylethylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(phenylpropylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(phenylbutylhydroxyphosphinyl)-2-phenylpropanoic acid;

20 3-((2,3,4-trimethoxyphenyl)-3-hydroxyphosphinyl)-2-phenylpropanoic acid;

3-(phenylprop-2-enylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-ethylpropanoic acid;

25 3-(benzylhydroxyphosphinyl)-2-propylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-butylpropanoic acid;

WO 98/13044

PCT/US97/14417

29

3- (benzylhydroxyphosphinyl) -2-cyclohexylpropanoic acid;
3- (benzylhydroxyphosphinyl) -2- (cyclohexyl)methylpropanoic
acid;
3- (benzylhydroxyphosphinyl) -2-phenylpropanoic acid;
5 3- (benzylhydroxyphosphinyl) -2-benzylpropanoic acid;
3- (benzylhydroxyphosphinyl) -2-phenylethylpropanoic acid;
3- (benzylhydroxyphosphinyl) -2-phenylpropylpropanoic acid;
3- (benzylhydroxyphosphinyl) -2-phenylbutylpropanoic acid;
3- (benzylhydroxyphosphinyl) -2- (2,3,4-trimethoxyphenyl) -
10 propanoic acid;
3- (benzylhydroxyphosphinyl) -2-phenylprop-2-enylpropanoic
acid; and
pharmaceutically acceptable salts and hydrates thereof.

In other embodiments, at least one of R₁ and R₂ is
15 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl,
tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-
thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, or C₁-C₄
straight or branched chain alkyl substituted with 2-
indolyl 3-indolyl, 4-indolyl, 2-furyl, 3-furyl,
20 tetrahydrofuranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-
pyridyl or 4-pyridyl; or R₁ is 1-naphthyl, 2-naphthyl, or
C₁-C₄ straight or branched chain alkyl substituted with
1-naphthyl or 2-naphthyl.

Preferred compounds of these embodiments include:

25 3- [(2-pyridyl)methylhydroxyphosphinyl] -2-phenylpropanoic
acid;

WO 98/13044

PCT/US97/14417

30

- 3-[(3-pyridyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(4-pyridyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 5 3-[(3-pyridyl)ethylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(3-pyridyl)propylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(tetrahydrofuranyl)methylhydroxyphosphinyl]-2-phenyl
- 10 propanoic acid;
- 3-[(tetrahydrofuranyl)ethylhydroxyphosphinyl]-2-phenyl propanoic acid;
- 3-[(tetrahydrofuranyl)propylhydroxyphosphinyl]-2-phenyl propanoic acid;
- 15 3-[(2-indolyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(3-indolyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(4-indolyl)methylhydroxyphosphinyl]-2-phenylpropanoic
- 20 acid;
- 3-[(3-indolyl)ethylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(3-indolyl)propylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 25 3-[(2-thienyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;

WO 98/13044

PCT/US97/14417

31

- 3-[(3-thienyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(4-thienyl)methylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 5 3-[(3-thienyl)ethylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-[(3-thienyl)propylhydroxyphosphinyl]-2-phenylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(2-pyridyl)methylpropanoic
- 10 acid;
- 3-(benzylhydroxyphosphinyl)-2-(3-pyridyl)methylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(4-pyridyl)methylpropanoic acid;
- 15 3-(benzylhydroxyphosphinyl)-2-(3-pyridyl)ethylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(3-pyridyl)propylpropanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(tetrahydrofuranyl)methyl
- 20 propanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(tetrahydrofuranyl)ethyl propanoic acid;
- 3-(benzylhydroxyphosphinyl)-2-(tetrahydrofuranyl)propyl propanoic acid;
- 25 3-(benzylhydroxyphosphinyl)-2-(2-indolyl)methylpropanoic acid;

WO 98/13044

PCT/US97/14417

32

3-(benzylhydroxyphosphinyl)-2-(3-indolyl)methylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-(4-indolyl)methylpropanoic acid;

5 3-(benzylhydroxyphosphinyl)-2-(3-indolyl)ethylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-(3-indolyl)propylpropanoic acid;

10 3-(benzylhydroxyphosphinyl)-2-(2-thienyl)methylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-(3-thienyl)methylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-(4-thienyl)methylpropanoic acid;

15 3-(benzylhydroxyphosphinyl)-2-(3-thienyl)ethylpropanoic acid;

3-(benzylhydroxyphosphinyl)-2-(3-thienyl)propylpropanoic acid;

3-((1-naphthyl)hydroxyphosphinyl)-2-phenylpropanoic acid;

20 3-((2-naphthyl)hydroxyphosphinyl)-2-phenylpropanoic acid;

3-((1-naphthyl)methylhydroxyphosphinyl)-2-phenylpropanoic acid;

3-((2-naphthyl)methylhydroxyphosphinyl)-2-phenylpropanoic acid;

25 3-((1-naphthyl)ethylhydroxyphosphinyl)-2-phenylpropanoic acid;

WO 98/13044

PCT/US97/14417

33

- 3 - ((2-naphthyl)ethylhydroxyphosphinyl) - 2-phenylpropanoic acid;
- 3 - ((1-naphthyl)propylhydroxyphosphinyl) - 2-phenylpropanoic acid;
- 5 3 - ((2-naphthyl)propylhydroxyphosphinyl) - 2-phenylpropanoic acid;
- 3 - ((1-naphthyl)butylhydroxyphosphinyl) - 2-phenylpropanoic acid;
- 3 - ((2-naphthyl)butylhydroxyphosphinyl) - 2-phenylpropanoic acid; and
- 10 pharmaceutically acceptable salts and hydrates thereof.

When Y is O, R₂ is preferably substituted with carboxy.

Exemplary compounds of this embodiment include:

- 15 2 - [[methylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[ethylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[propylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[butylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[cyclohexylhydroxyphosphinyl]oxy]pentanedioic acid;
- 20 2 - [[(cyclohexyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[phenylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[benzylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[phenylethylhydroxyphosphinyl]oxy]pentanedioic acid;
- 25 2 - [[phenylpropylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[phenylbutylhydroxyphosphinyl]oxy]pentanedioic acid;

WO 98/13044

PCT/US97/14417

34

- 2 - [[(4-methylbenzyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(4-fluorobenzyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 5 2 - [[(2-fluorobenzyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(pentafluorobenzyl)hydroxyphosphinyl]oxy] pentanedioic acid;
- 2 - [[(methoxybenzyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 10 2 - [[(2,3,4-trimethoxyphenyl)hydroxyphosphinyl]oxy] - pentanedioic acid;
- 2 - [[(1-naphthyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(2-naphthyl)hydroxyphosphinyl]oxy]pentanedioic acid;
- 15 2 - [[(1-naphthyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(2-naphthyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(1-naphthyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;
- 20 2 - [[(2-naphthyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2 - [[(1-naphthyl)propylhydroxyphosphinyl]oxy]pentanedioic acid;
- 25 2 - [[(2-naphthyl)propylhydroxyphosphinyl]oxy]pentanedioic acid;

WO 98/13044

PCT/US97/14417

35

- 2-[[(1-naphthyl) butylhydroxyphosphinyl] oxy] pentanedioic acid;
- 2-[[(2-naphthyl) butylhydroxyphosphinyl] oxy] pentanedioic acid;
- 5 2-[[(phenylprop-2-enyl) hydroxyphosphinyl] oxy] pentanedioic acid;
- 2-[[benzylhydroxyphosphinyl] oxy] pentanedioic acid;
- 2-[[(hydroxy) phenylmethyl] hydroxyphosphinyl] oxy] pentanedioic acid;
- 10 2-[[(3-methylbenzyl) hydroxyphosphinyl] oxy] pentanedioic acid;
- 2-[[(4-fluorophenyl) hydroxyphosphinyl] oxy] pentanedioic acid;
- 2-[[(2-fluorobenzyl) hydroxyphosphinyl] oxy] pentanedioic acid;
- 15 2- (phosphono) oxy] pentanedioic acid;
- 2-[[(3-trifluoromethylbenzyl) hydroxyphosphinyl] oxy] - pentanedioic acid;
- 2-[[methylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;
- 20 2-[[ethylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;
- 2-[[propylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;
- 2-[[butylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;
- 2-[[cyclohexylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;
- 25 2-[[(cyclohexyl) methylhydroxyphosphinyl] oxy] -2-phenylethanoic acid;

WO 98/13044

PCT/US97/14417

36

- 2- [[phenylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
2- [[benzylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
2- [[phenylethylhydroxyphosphinyl]oxy]-2-phenylethanoic
acid;
5 2- [[phenylpropylhydroxyphosphinyl]oxy]-2-phenylethanoic
acid;
2- [[phenylbutylhydroxyphosphinyl]oxy]-2-phenylethanoic
acid;
2- [[(2,3,4-trimethoxyphenyl)-3-hydroxyphosphinyl]oxy]-2-
10 phenylethanoic acid;
2- [[(1-naphthyl)hydroxyphosphinyl]oxy]-2-phenylethanoic
acid;
2- [[(2-naphthyl)hydroxyphosphinyl]oxy]-2-phenylethanoic
acid;
15 2- [[(1-naphthyl)methylhydroxyphosphinyl]oxy]-2-phenyl-
ethanoic acid;
2- [[(2-naphthyl)methylhydroxyphosphinyl]oxy]-2-phenyl-
ethanoic acid;
2- [[(1-naphthyl)ethylhydroxyphosphinyl]oxy]-2-phenyl-
20 ethanoic acid;
2- [[(2-naphthyl)ethylhydroxyphosphinyl]oxy]-2-phenyl-
ethanoic acid;
2- [[(1-naphthyl)propylhydroxyphosphinyl]oxy]-2-phenyl-
ethanoic acid;
25 2- [[(2-naphthyl)propylhydroxyphosphinyl]oxy]-2-phenyl-
ethanoic acid;

WO 98/13044

PCT/US97/14417

37

- 2-[[(1-naphthyl)butylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[(2-naphthyl)butylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 5 2-[[phenylprop-2-enylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[(methylhydroxyphosphinyl)oxy]hexanedioic acid;
- 2-[(benzylhydroxyphosphinyl)oxy]hexanedioic acid;
- 2-[(methylhydroxyphosphinyl)oxy]heptanedioic acid;
- 10 2-[(benzylhydroxyphosphinyl)oxy]heptanedioic acid;
- 2-[(methylhydroxyphosphinyl)oxy]octanedioic acid;
- 2-[(benzylhydroxyphosphinyl)oxy]octanedioic acid;
- 2-[(methylhydroxyphosphinyl)oxy]nonanedioic acid;
- 2-[(benzylhydroxyphosphinyl)oxy]nonanedioic acid;
- 15 2-[(methylhydroxyphosphinyl)oxy]decanedioic acid;
- 2-[(benzylhydroxyphosphinyl)oxy]decanedioic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-methylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-ethylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-propylethanoic acid;
- 20 2-[[benzylhydroxyphosphinyl]oxy]-2-butylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-cyclohexylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(cyclohexyl)methyl-ethanoic acid;
- 25 2-[[benzylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-benzylethanoic acid;

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

38

- 2-[[benzylhydroxyphosphinyl]oxy]-2-phenylethylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-phenylpropylethanoic acid;
- 5 2-[[benzylhydroxyphosphinyl]oxy]-2-phenylbutylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(2,3,4-trimethoxyphenyl)ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(1-naphthyl)ethanoic
- 10 acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-naphthyl)ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(1-naphthyl)methyl-ethanoic acid;
- 15 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-naphthyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(1-naphthyl)ethyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-naphthyl)ethyl-
- 20 ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(1-naphthyl)propyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-naphthyl)propyl-ethanoic acid;
- 25 2-[[benzylhydroxyphosphinyl]oxy]-2-(1-naphthyl)butyl-ethanoic acid;

WO 98/13044

PCT/US97/14417

39

2-[[benzylhydroxyphosphinyl]oxy]-2-(2-naphthyl)butyl-ethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-phenylprop-2-enyl-ethanoic acid;

5 2-[[(2-pyridyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(3-pyridyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

10 2-[[(4-pyridyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(3-pyridyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(3-pyridyl)propylhydroxyphosphinyl]oxy]pentanedioic acid;

15 2-[[(tetrahydrofuranyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(tetrahydrofuranyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;

20 2-[[(tetrahydrofuranyl)propylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(2-indolyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[(3-indolyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

25 2-[[(4-indolyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

WO 98/13044

PCT/US97/14417

40

2-[[[(3-indolyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[[(3-indolyl)propylhydroxyphosphinyl]oxy]pentanedioic acid;

5 2-[[[(2-thienyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[[(3-thienyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

10 2-[[[(4-thienyl)methylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[[(3-thienyl)ethylhydroxyphosphinyl]oxy]pentanedioic acid;

2-[[[(3-thienyl)propylhydroxyphosphinyl]oxy]pentanedioic acid; and

15 pharmaceutically acceptable salts and hydrates thereof.

In another preferred embodiment, R_2 is selected from the group consisting of hydrogen, C_1-C_9 straight or branched chain alkyl, C_2-C_9 straight or branched chain alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, benzyl and
20 phenyl, wherein said R_2 is unsubstituted or substituted with C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl, C_1-C_6 alkoxy, phenyl or mixtures thereof.

Exemplary compounds of this embodiment include:

25 2-[[[(2-pyridyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

WO 98/13044

PCT/US97/14417

41

- 2-[[[(3-pyridyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(4-pyridyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 5 2-[[[(3-pyridyl)ethylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(3-pyridyl)propylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(tetrahydrofuranyl)methylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
- 10 2-[[[(tetrahydrofuranyl)ethylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
- 2-[[[(tetrahydrofuranyl)propylhydroxyphosphinyl]oxy]-2-phenylethanoic acid;
- 15 2-[[[(2-indolyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(3-indolyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(4-indolyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 20 2-[[[(3-indolyl)ethylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 2-[[[(3-indolyl)propylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;
- 25 2-[[[(2-thienyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

WO 98/13044

PCT/US97/14417

42

2-[[(3-thienyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

2-[[(4-thienyl)methylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

5 2-[[(3-thienyl)ethylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

2-[[(3-thienyl)propylhydroxyphosphinyl]oxy]-2-phenyl-ethanoic acid;

10 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-pyridyl)methyl-ethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-(3-pyridyl)methyl-ethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-(4-pyridyl)methyl-ethanoic acid;

15 2-[[benzylhydroxyphosphinyl]oxy]-2-(3-pyridyl)ethyl-ethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-(3-pyridyl)propyl-ethanoic acid;

20 2-[[benzylhydroxyphosphinyl]oxy]-2-(tetrahydrofuranyl)-methylethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-(tetrahydrofuranyl)-ethylethanoic acid;

2-[[benzylhydroxyphosphinyl]oxy]-2-(tetrahydrofuranyl)-propylethanoic acid;

25 2-[[benzylhydroxyphosphinyl]oxy]-2-(2-indolyl)methyl-ethanoic acid;

WO 98/13044

PCT/US97/14417

43

- 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-indolyl)methyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl]oxy]-2-(4-indolyl)methyl-ethanoic acid;
- 5 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-indolyl)ethyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-indolyl)propyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl]oxy]-2-(2-thienyl)methyl-ethanoic acid;
- 10 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-thienyl)methyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl]oxy]-2-(4-thienyl)methyl-ethanoic acid;
- 15 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-thienyl)ethyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl]oxy]-2-(3-thienyl)propyl-ethanoic acid; and
- pharmaceutically acceptable salts and hydrates thereof.

- 20 When Y is NR_5 , R_2 is preferably substituted with carboxy.

Exemplary compounds of this embodiment include:

- 2- [[methylhydroxyphosphinyl]amino]pentanedioic acid;
- 2- [[ethylhydroxyphosphinyl]amino]pentanedioic acid;
- 25 2- [[propylhydroxyphosphinyl]amino]pentanedioic acid;
- 2- [[butylhydroxyphosphinyl]amino]pentanedioic acid;

WO 98/13044

PCT/US97/14417

44

- 2- [[cyclohexylhydroxyphosphinyl] amino] pentanedioic acid;
2- [[[cyclohexyl)methylhydroxyphosphinyl] amino] pentane-
dioic acid;
2- [[phenylhydroxyphosphinyl] amino] pentanedioic acid;
5 2- [[benzylhydroxyphosphinyl] amino] pentanedioic acid;
2- [[phenylethylhydroxyphosphinyl] amino] pentanedioic acid;
2- [[phenylpropylhydroxyphosphinyl] amino] pentanedioic
acid;
2- [[phenylbutylhydroxyphosphinyl] amino] pentanedioic acid;
10 2- [[[4-methylbenzyl) hydroxyphosphinyl] amino] pentanedioic
acid;
2- [[[4-fluorobenzyl) hydroxyphosphinyl] amino] pentanedioic
acid;
2- [[[2-fluorobenzyl) hydroxyphosphinyl] amino] pentanedioic
15 acid;
2- [[[pentafluorobenzyl) hydroxyphosphinyl] amino] pentane-
dioic acid;
2- [[[methoxybenzyl) hydroxyphosphinyl] amino] pentanedioic
acid;
20 2- [[[2,3,4-trimethoxyphenyl) hydroxyphosphinyl] amino] -
pentanedioic acid;
2- [[[1-naphthyl) hydroxyphosphinyl] amino] pentanedioic
acid;
2- [[[2-naphthyl) hydroxyphosphinyl] amino] pentanedioic
25 acid;
2- [[[1-naphthyl) methylhydroxyphosphinyl] amino] pentane-

WO 98/13044

PCT/US97/14417

45

dioic acid;

2-[[(2-naphthyl)methylhydroxyphosphinyl]amino]pentane-
dioic acid;

2-[[(1-naphthyl)ethylhydroxyphosphinyl]amino]pentanedioic
5 acid;

2-[[(2-naphthyl)ethylhydroxyphosphinyl]amino]pentanedioic
acid;

2-[[(1-naphthyl)propylhydroxyphosphinyl]amino]pentane-
dioic acid;

10 2-[[(2-naphthyl)propylhydroxyphosphinyl]amino]pentane-
dioic acid;

2-[[(1-naphthyl)butylhydroxyphosphinyl]amino]pentanedioic
acid;

2-[[(2-naphthyl)butylhydroxyphosphinyl]amino]pentanedioic
15 acid;

2-[[(phenylprop-2-enyl)hydroxyphosphinyl]amino]pentane-
dioic acid;

2-[[(benzylhydroxyphosphinyl)amino]pentanedioic acid;

2-[[(2-fluorobenzyl)hydroxyphosphinyl]amino]-2-pentane-
20 dioic acid;

2-[[((hydroxy)phenylmethyl)hydroxyphosphinyl]amino] -
pentanedioic acid;

2-[[(3-methylbenzyl)hydroxyphosphinyl]amino]pentanedioic
acid;

25 2-[[(4-fluorophenyl)hydroxyphosphinyl]amino]pentanedioic
acid;

WO 98/13044

PCT/US97/14417

46

- 2- [(phosphono)amino]pentanedioic acid;
2- [[(3-trifluoromethylbenzyl)hydroxyphosphinyl]amino] -
pentanedioic acid;
2- [(methylhydroxyphosphinyl)amino]hexanedioic acid;
5 2- [(benzylhydroxyphosphinyl)amino]hexanedioic acid;
2- [(methylhydroxyphosphinyl)amino]heptanedioic acid;
2- [(benzylhydroxyphosphinyl)amino]heptanedioic acid;
2- [(methylhydroxyphosphinyl)amino]octanedioic acid;
2- [(benzylhydroxyphosphinyl)amino]octanedioic acid;
10 2- [(methylhydroxyphosphinyl)amino]nonanedioic acid;
2- [(benzylhydroxyphosphinyl)amino]nonanedioic acid;
2- [(methylhydroxyphosphinyl)amino]decanedioic acid;
2- [(benzylhydroxyphosphinyl)amino]decanedioic acid;
15 3- [[(2-pyridyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;
3- [[(3-pyridyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;
3- [[(4-pyridyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;
20 3- [[(3-pyridyl)ethylhydroxyphosphinyl]amino]pentanedioic
acid;
3- [[(3-pyridyl)propylhydroxyphosphinyl]amino]pentanedioic
acid;
3- [[(tetrahydrofuranyl)methylhydroxyphosphinyl]amino] -
25 pentanedioic acid;
3- [[(tetrahydrofuranyl)ethylhydroxyphosphinyl]amino] -

WO 98/13044

PCT/US97/14417

47

pentanedioic acid;

3-[[[(tetrahydrofuranyl)propylhydroxyphosphinyl]amino]-
pentanedioic acid;

3-[[[(2-indolyl)methylhydroxyphosphinyl]amino]pentanedioic
5 acid;

3-[[[(3-indolyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;

3-[[[(4-indolyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;

10 3-[[[(3-indolyl)ethylhydroxyphosphinyl]amino]pentanedioic
acid;

3-[[[(3-indolyl)propylhydroxyphosphinyl]amino]pentanedioic
acid;

3-[[[(2-thienyl)methylhydroxyphosphinyl]amino]pentanedioic
15 acid;

3-[[[(3-thienyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;

3-[[[(4-thienyl)methylhydroxyphosphinyl]amino]pentanedioic
acid;

20 3-[[[(3-thienyl)ethylhydroxyphosphinyl]amino]pentanedioic
acid;

3-[[[(3-thienyl)propylhydroxyphosphinyl]amino]pentanedioic
acid; and

pharmaceutically acceptable salts and hydrates thereof.

25 In another preferred embodiment, R₂ is selected from
the group consisting of hydrogen, C₁-C₉, straight or

WO 98/13044

PCT/US97/14417

48

branched chain alkyl, C₂-C₉, straight or branched chain alkenyl, C₃-C₈, cycloalkyl, C₅-C₇, cycloalkenyl, benzyl and phenyl, wherein said R₂ is unsubstituted or substituted with C₃-C₈, cycloalkyl, C₅-C₇, cycloalkenyl, C₁-C₆, straight or branched chain alkyl, C₂-C₆, straight or branched chain alkenyl, C₁-C₄, alkoxy, phenyl or mixtures thereof.

Exemplary compounds of this embodiment include:

- 2-[[methylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[ethylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
10 2-[[propylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[butylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[cyclohexylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[cyclohexyl)methylhydroxyphosphinyl]amino]-2-phenyl-
15 ethanoic acid;
2-[[phenylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[benzylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[phenylethylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
20 2-[[phenylpropylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[phenylbutylhydroxyphosphinyl]amino]-2-phenylethanoic acid;
2-[[2,3,4-trimethoxyphenyl]-3-hydroxyphosphinyl]amino]-
25 2-phenylethanoic acid;
2-[[1-naphthyl]hydroxyphosphinyl]amino]-2-phenylethanoic

WO 98/13044

PCT/US97/14417

49

acid;

2-[[[(2-naphthyl)hydroxyphosphinyl]amino]-2-phenylethanoic
acid;

2-[[[(1-naphthyl)methylhydroxyphosphinyl]amino]-2-phenyl-
5 ethanoic acid;

2-[[[(2-naphthyl)methylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

2-[[[(1-naphthyl)ethylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

10 2-[[[(2-naphthyl)ethylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

2-[[[(1-naphthyl)propylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

2-[[[(2-naphthyl)propylhydroxyphosphinyl]amino]-2-phenyl-
15 ethanoic acid;

2-[[[(1-naphthyl)butylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

2-[[[(2-naphthyl)butylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

20 2-[[[phenylprop-2-enylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;

2-[[[benzylhydroxyphosphinyl]amino]-2-methylethanoic acid;

2-[[[benzylhydroxyphosphinyl]amino]-2-ethylethanoic acid;

2-[[[benzylhydroxyphosphinyl]amino]-2-propylethanoic acid;

25 2-[[[benzylhydroxyphosphinyl]amino]-2-butylethanoic acid;

2-[[[benzylhydroxyphosphinyl]amino]-2-cyclohexylethanoic

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

50

- acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (cyclohexyl) methyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- phenylethanoic acid;
- 5 2- [[benzylhydroxyphosphinyl] amino] -2- benzylethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- phenylethylethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- phenylpropylethanoic acid;
- 10 2- [[benzylhydroxyphosphinyl] amino] -2- phenylbutylethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (2,3,4-trimethoxyphenyl) ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (1-naphthyl) ethanoic acid;
- 15 acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (2-naphthyl) ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (1-naphthyl) methyl-ethanoic acid;
- 20 2- [[benzylhydroxyphosphinyl] amino] -2- (2-naphthyl) methyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (1-naphthyl) ethyl-ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (2-naphthyl) ethyl-ethanoic acid;
- 25 ethanoic acid;
- 2- [[benzylhydroxyphosphinyl] amino] -2- (1-naphthyl) propyl-

WO 98/13044

PCT/US97/14417

51

- ethanoic acid;
2- [[benzylhydroxyphosphinyl] amino] -2- (2-naphthyl) propyl-
ethanoic acid;
2- [[benzylhydroxyphosphinyl] amino] -2- (1-naphthyl) butyl-
5 ethanoic acid;
2- [[benzylhydroxyphosphinyl] amino] -2- (2-naphthyl) butyl-
ethanoic acid;
2- [[benzylhydroxyphosphinyl] amino] -2-phenolprop-2-enyl-
ethanoic acid;
10 2- [[(2-pyridyl)methylhydroxyphosphinyl] amino] -2-phenyl-
ethanoic acid;
2- [[(3-pyridyl)methylhydroxyphosphinyl] amino] -2-phenyl-
ethanoic acid;
2- [[(4-pyridyl)methylhydroxyphosphinyl] amino] -2-phenyl-
15 ethanoic acid;
2- [[(3-pyridyl)ethylhydroxyphosphinyl] amino] -2-phenyl-
ethanoic acid;
2- [[(3-pyridyl)propylhydroxyphosphinyl] amino] -2-phenyl-
ethanoic acid;
20 2- [[(tetrahydrofuranyl)methylhydroxyphosphinyl] amino] -2-
phenylethanoic acid;
2- [[(tetrahydrofuranyl)ethylhydroxyphosphinyl] amino] -2-
phenylethanoic acid;
2- [[(tetrahydrofuranyl)propylhydroxyphosphinyl] amino] -2-
25 phenylethanoic acid;
2- [[(2-indolyl)methylhydroxyphosphinyl] amino] -2-phenyl-

WO 98/13044

PCT/US97/14417

52

- ethanoic acid;
2-[[[(3-indolyl)methylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
2-[[[(4-indolyl)methylhydroxyphosphinyl]amino]-2-phenyl-
5 ethanoic acid;
2-[[[(3-indolyl)ethylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
2-[[[(3-indolyl)propylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
10 2-[[[(2-thienyl)methylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
2-[[[(3-thienyl)methylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
2-[[[(4-thienyl)methylhydroxyphosphinyl]amino]-2-phenyl-
15 ethanoic acid;
2-[[[(3-thienyl)ethylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
2-[[[(3-thienyl)propylhydroxyphosphinyl]amino]-2-phenyl-
ethanoic acid;
20 2-[[[benzylhydroxyphosphinyl]amino]-2-(2-pyridyl)methyl-
ethanoic acid;
2-[[[benzylhydroxyphosphinyl]amino]-2-(3-pyridyl)methyl-
ethanoic acid;
2-[[[benzylhydroxyphosphinyl]amino]-2-(4-pyridyl)methyl-
25 ethanoic acid;
2-[[[benzylhydroxyphosphinyl]amino]-2-(3-pyridyl)ethyl-

WO 98/13044

PCT/US97/14417

53

- ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-pyridyl)propyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(tetrahydrofuranyl)-methylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(tetrahydrofuranyl)-ethylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(tetrahydrofuranyl)-propylethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(2-indolyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-indolyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(4-indolyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-indolyl)ethyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-indolyl)propyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(2-thienyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-thienyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(4-thienyl)methyl-ethanoic acid;
- 2-[[benzylhydroxyphosphinyl]amino]-2-(3-thienyl)ethyl-

WO 98/13044

PCT/US97/14417

54

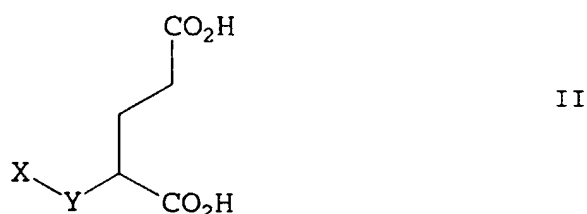
ethanoic acid;

2-[[benzylhydroxyphosphinyl]amino]-2-(3-thienyl)propyl-

ethanoic acid; and

pharmaceutically acceptable salts and hydrates thereof.

5 Another preferred NAALADase inhibitor is a compound
of formula II:

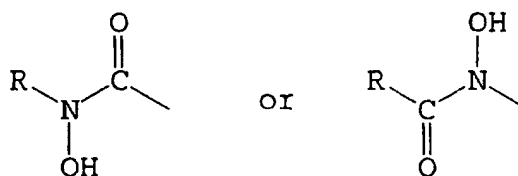


10

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

X is

15



III

IV ;

20

Y is CR₁R₂, NR₃ or O;

R, R₁, R₂ and R₃ are independently selected from the
group consisting of hydrogen, C₁-C₈ straight or branched
chain alkyl, C₂-C₈ straight or branched chain alkenyl, C₃-
25 C₈ cycloalkyl, C₅-C₇ cycloalkenyl, Ar and mixtures
thereof, wherein said R, R₁, R₂ and R₃ are independently

WO 98/13044

PCT/US97/14417

55

unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

In a preferred embodiment, Y is CH₂.

In a more preferred embodiment, R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-pyridyl, benzyl and phenyl, said R having one to three substituent(s) independently selected from the group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

In the most preferred embodiment, the compound is

WO 98/13044

PCT/US97/14417

56

selected from the group consisting of:

2- [[(N-hydroxy) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy-N-methyl) carbamoyl] methyl] pentanedioic acid;

5 2- [[(N-butyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

2- [[(N-benzyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

10 2- [[(N-hydroxy-N-phenyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy-N-2-phenylethyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-ethyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

15 2- [[(N-hydroxy-N-propyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy-N-3-phenylpropyl) carbamoyl] methyl] pentanedioic acid;

20 2- [[(N-hydroxy-N-4-pyridyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy) carboxamido] methyl] pentanedioic acid;

2- [[N-hydroxy (methyl) carboxamido] methyl] pentanedioic acid;

25 2- [[N-hydroxy (benzyl) carboxamido] methyl] pentanedioic acid;

2- [[N-hydroxy (phenyl) carboxamido] methyl] pentanedioic

WO 98/13044

PCT/US97/14417

57

acid;

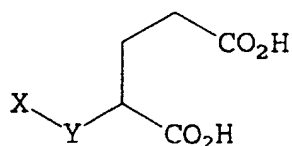
2- [[N-hydroxy (2-phenylethyl) carboxamido] methyl] pentane-
dioic acid;

2- [[N-hydroxy (ethyl) carboxamido] methyl] pentanedioic acid;

5 2- [[N-hydroxy (propyl) carboxamido] methyl] pentanedioic
acid;2- [[N-hydroxy (3-phenylpropyl) carboxamido] methyl] pentane-
dioic acid; and10 2- [[N-hydroxy (4-pyridyl) carboxamido] methyl] pentanedioic
acid.

Another preferred NAALADase inhibitor is a compound
of formula V:

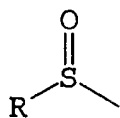
15



V

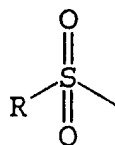
or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

20 X is selected from the group consisting of



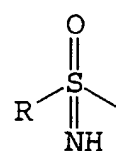
VI

and



VII

and



VIII

;

25

WO 98/13044

PCT/US97/14417

58

Y is CR₁R₂, NR₃ or O;

R, R₁, R₂ and R₃ are independently selected from the group consisting of hydrogen, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R, R₁, R₂ and R₃ are independently unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, said Ar having one to three substituent(s) independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino and mixtures thereof.

In a preferred embodiment, at least one of said R, R₁, R₂ and R₃ is/are independently substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, hydroxy, halo, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-

WO 98/13044

PCT/US97/14417

59

C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof.

In a more preferred embodiment, Y is CH₂.

In an even more preferred embodiment, R is selected
5 from the group consisting of hydrogen, C₁-C₄ straight or
branched chain alkyl, 4-pyridyl, benzyl and phenyl, said
R having one to three substituent(s) independently
selected from the group consisting of hydrogen, C₃-C₈
cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro,
10 trifluoromethyl, C₁-C₆ straight or branched chain alkyl,
C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-
C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures
thereof.

In the most preferred embodiment, the compound is
15 selected from the group consisting of:

2-[(sulfinyl)methyl]pentanedioic acid;
2-[(methylsulfinyl)methyl]pentanedioic acid;
2-[(ethylsulfinyl)methyl]pentanedioic acid;
2-[(propylsulfinyl)methyl]pentanedioic acid;
20 2-[(butylsulfinyl)methyl]pentanedioic acid;
2-[(phenylsulfinyl)methyl]pentanedioic acid;
2-[[(2-phenylethyl)sulfinyl]methyl]pentanedioic acid;
2-[[(3-phenylpropyl)sulfinyl]methyl]pentanedioic acid;
2-[[(4-pyridyl)sulfinyl]methyl]pentanedioic acid;
25 2-[(benzylsulfinyl)methyl]pentanedioic acid;
2-[(sulfonyl)methyl]pentanedioic acid;

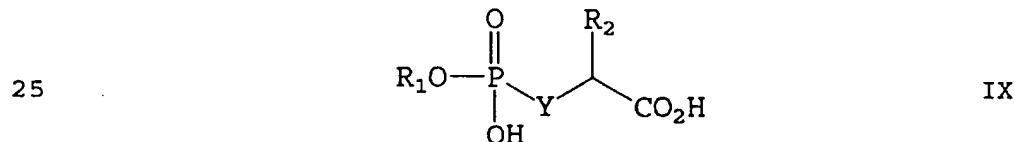
WO 98/13044

PCT/US97/14417

60

- 2-[(methylsulfonyl)methyl]pentanedioic acid;
2-[(ethylsulfonyl)methyl]pentanedioic acid;
2-[(propylsulfonyl)methyl]pentanedioic acid;
2-[(butylsulfonyl)methyl]pentanedioic acid;
5 2-[(phenylsulfonyl)methyl]pentanedioic acid;
2-[[(2-phenylethyl)sulfonyl]methyl]pentanedioic acid;
2-[[(3-phenylpropyl)sulfonyl]methyl]pentanedioic acid;
2-[[(4-pyridyl)sulfonyl]methyl]pentanedioic acid; and
2-[(benzylsulfonyl)methyl]pentanedioic acid;
10 2-[(sulfoximinyl)methyl]pentanedioic acid;
2-[(methylsulfoximinyl)methyl]pentanedioic acid;
2-[(ethylsulfoximinyl)methyl]pentanedioic acid;
2-[(propylsulfoximinyl)methyl]pentanedioic acid;
2-[(butylsulfoximinyl)methyl]pentanedioic acid;
15 2-[(phenylsulfoximinyl)methyl]pentanedioic acid;
2-[[(2-phenylethyl)sulfoximinyl]methyl]pentanedioic acid;
2-[[(3-phenylpropyl)sulfoximinyl]methyl]pentanedioic
acid;
2-[[(4-pyridyl)sulfoximinyl]methyl]pentanedioic acid; and
20 2-[(benzylsulfoximinyl)methyl]pentanedioic acid.

Another preferred NAALADase inhibitor is a compound
of formula IX:



WO 98/13044

PCT/US97/14417

61

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

Y is CR₃R₄, NR₅ or O;

R₂ is selected from the group consisting of
5 hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉
straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
C₇ cycloalkenyl and Ar, wherein said R₂ is unsubstituted
or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
10 C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy,
phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₁, R₃, R₄ and R₅ are independently selected from the
group consisting of hydrogen, C₁-C₉ straight or branched
15 chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-
C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁,
R₂ and R₃ are independently unsubstituted or
substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl,
halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or
20 branched chain alkyl, C₂-C₆ straight or branched chain
alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-
naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-
25 furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-
pyridyl, benzyl and phenyl, wherein said Ar has one to

WO 98/13044

PCT/US97/14417

62

three substituent(s) independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-
5 C₆ alkenyloxy, phenoxy, benzyloxy, amino and mixtures thereof.

In a preferred embodiment, Y is CH₂.

When R is hydrogen, the compound is preferably selected from the group consisting of:

- 10 phosphonopropanoic acid;
- 2-methyl-3-phosphonopropanoic acid;
- 2-ethyl-3-phosphonopropanoic acid;
- 2-propyl-3-phosphonopropanoic acid;
- 2-butyl-3-phosphonopropanoic acid;
- 15 2-phenyl-3-phosphonopropanoic acid;
- 2-(2-phenylethyl)-3-phosphonopropanoic acid;
- 2-(3-phenylpropyl)-3-phosphonopropanoic acid;
- 2-(4-pyridyl)-3-phosphonopropanoic acid; and
- 2-benzyl-3-phosphonopropanoic acid.

- 20 When R₂ is substituted with carboxy, the compound is selected from the group consisting of:

- 2-(hydrohydroxyphosphonomethyl)pentanedioic acid;
- 2-(hydromethoxyphosphonomethyl)pentanedioic acid;
- 2-(hydroethoxyphosphonomethyl)pentanedioic acid;
- 25 2-(hydropropoxyphosphonomethyl)pentanedioic acid;
- 2-(hydrobutoxyphosphonomethyl)pentanedioic acid;

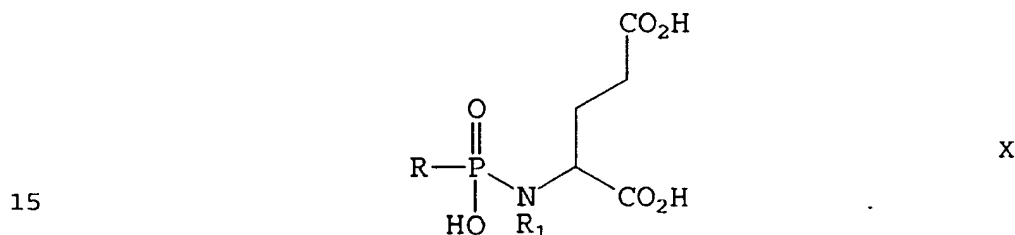
WO 98/13044

PCT/US97/14417

63

2- (hydrophenoxyphosphonomethyl)pentanedioic acid;
2- [hydro (2-phenylethoxy) phosphonomethyl]pentanedioic
acid;
2- [hydro (3-phenylpropoxy) phosphonomethyl]pentanedioic
5 acid;
2- [hydro (4-pyridyloxy) phosphonomethyl]pentanedioic acid;
and
2- (hydrobenzyloxyphosphonomethyl)pentanedioic acid.

Another preferred NAALADase inhibitor is a compound
10 of formula X:



or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

R and R₁ are independently selected from the group
20 consisting of hydrogen, C₁-C₈ straight or branched chain
alkyl or alkenyl group, C₃-C₈ cycloalkyl, C₃ or C₅
cycloalkyl, C₅-C₈ cycloalkenyl and Ar, wherein said R and
R₁ are independently unsubstituted or substituted with
C₃-C₈ cycloalkyl, C₅-C₈ cycloalkenyl, halo, hydroxy,
25 nitro, trifluoromethyl, C₁-C₆ straight or branched chain
alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₈

WO 98/13044

PCT/US97/14417

64

alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

In a preferred embodiment, the compound is selected from the group consisting of:

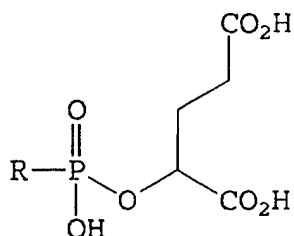
N-[methylhydroxyphosphinyl]glutamic acid;
N-[ethylhydroxyphosphinyl]glutamic acid;
N-[propylhydroxyphosphinyl]glutamic acid;
N-[butylhydroxyphosphinyl]glutamic acid;
N-[phenylhydroxyphosphinyl]glutamic acid;
N-[(phenylmethyl)hydroxyphosphinyl]glutamic acid;
N-[((2-phenylethyl)methyl)hydroxyphosphinyl]glutamic acid; and
N-methyl-N-[phenylhydroxyphosphinyl]glutamic acid.

A final preferred NAALADase inhibitor is a compound of formula XI:

WO 98/13044

PCT/US97/14417

65



XI

5

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

10 R is selected from the group consisting of hydrogen,
C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or
branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, Ar and mixtures thereof, wherein said R is
unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇
15 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy,
phenoxy, benzyloxy, amino, Ar or a mixture thereof;

Ar is selected from the group consisting of 1-
20 naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-
furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or
phenyl, having one to three substituents which are
independently selected from the group consisting of
hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C₁-C₆
25 straight or branched alkyl or alkenyl, C₁-C₆ alkoxy or C₁-
C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

WO 98/13044

PCT/US97/14417

66

In a preferred embodiment, the compound is selected from the group consisting of:

- 2-[[methylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2-[[ethylhydroxyphosphinyl]oxy]pentanedioic acid;
- 5 2-[[propylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2-[[butylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2-[[phenylhydroxyphosphinyl]oxy]pentanedioic acid;
- 2-[[[(4-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic acid;
- 10 2-[[[(2-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic acid;
- 2-[[[(phenylmethyl)hydroxyphosphinyl]oxy]pentanedioic acid; and
- 2-[[[(2-phenylethyl)methyl]hydroxyphosphinyl]oxy]-
- 15 pentanedioic acid.

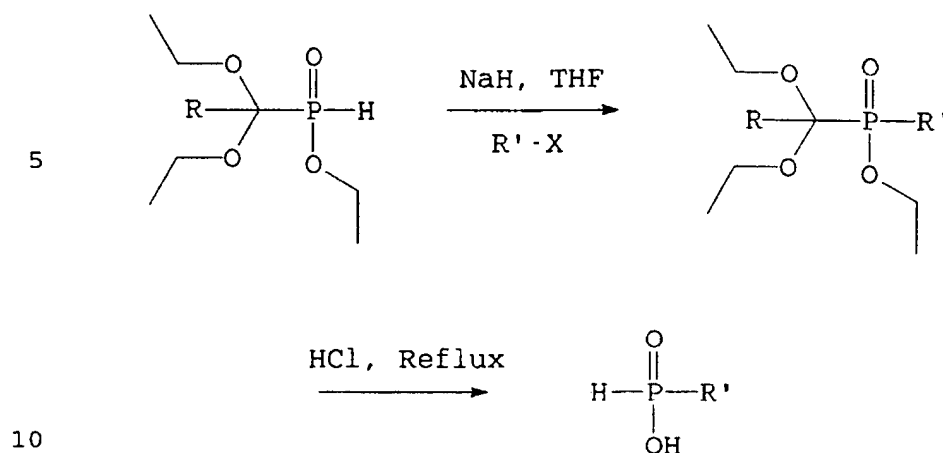
Synthesis of NAALADase Inhibitors

The NAALADase inhibitors of formula I can be readily prepared by standard techniques of organic chemistry, utilizing the general synthetic pathways depicted below in Schemes I-IX. Precursor compounds can be prepared by methods known in the art, such as those described by Jackson et al., *J. Med. Chem.*, Vol. 39, No. 2, pp. 619-622 (1996) and Froestl et al., *J. Med. Chem.*, Vol. 38, pp. 3313-3331 (1995).

WO 98/13044

PCT/US97/14417

67

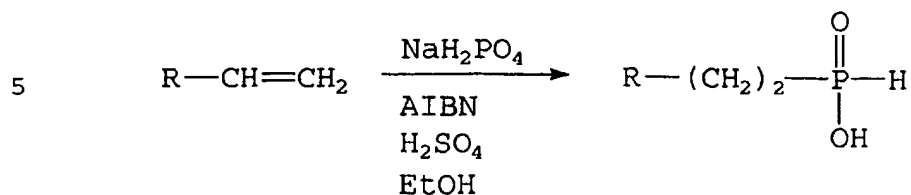
Scheme I

Methods of substituting the R group are known in the art. Additional methods of synthesizing phosphinic acid esters are described in *J. Med. Chem.*, Vol. 31, pp. 204-212 (1988), and set forth below in Scheme II.

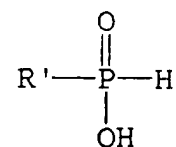
WO 98/13044

PCT/US97/14417

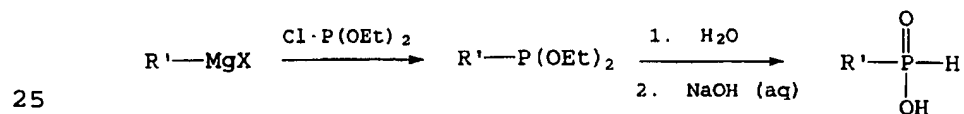
68

Scheme II**Method A**

10



- | | | | |
|-------|--|----|---|
| A. | $\text{R}' = (\text{CH}_2)_3\text{Ph}$ | H. | $\text{R}' = n\text{-C}_7\text{H}_{15}$ |
| 15 B. | $(\text{CH}_2)_4\text{Ph}$ | I. | $n\text{-C}_8\text{H}_{17}$ |
| C. | $(\text{CH}_2)_5\text{Ph}$ | J. | $n\text{-C}_9\text{H}_{19}$ |
| D. | $(\text{CH}_2)_4(\text{P-F-Ph})$ | K. | $\text{CH}_2\text{CHCH}_3\text{C}_4\text{H}_9$ |
| E. | $(\text{CH}_2)_4-(3\text{-pyridyl})$ | L. | $\text{CH}_2(\text{CH}_3)\text{C}(\text{CH}_3)_2$ |
| F. | $n\text{-C}_5\text{H}_{11}$ | | |
| 20 G. | $n\text{-C}_6\text{H}_{13}$ | | |

Method B

WO 98/13044

PCT/US97/14417

69

N. $R' = n-C_4H_9$ O. $CHCH_3C_5H_{11}$

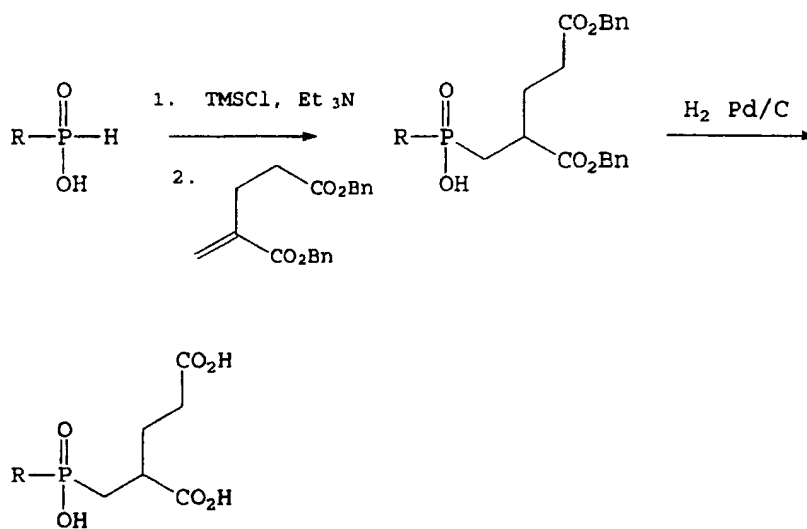
Starting with the aforementioned phosphinic acid
5 esters, there are a variety of routes for preparing the
compounds of formula I. For example, a general route has
been described in *J. Med. Chem.*, Vol. 39, pp. 619-622
(1996), and is set forth below in Scheme III.

10

Scheme III

15

20



Other routes for preparing the compounds of formula
I are set forth below in Scheme IV and Scheme V. Scheme
25 IV and Scheme V show the starting material as a
phosphinic acid derivative and the R group as any

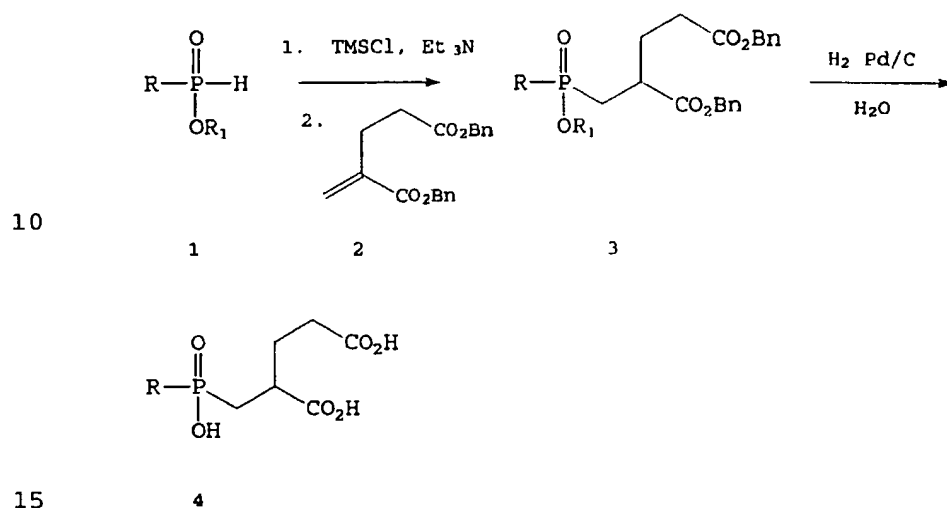
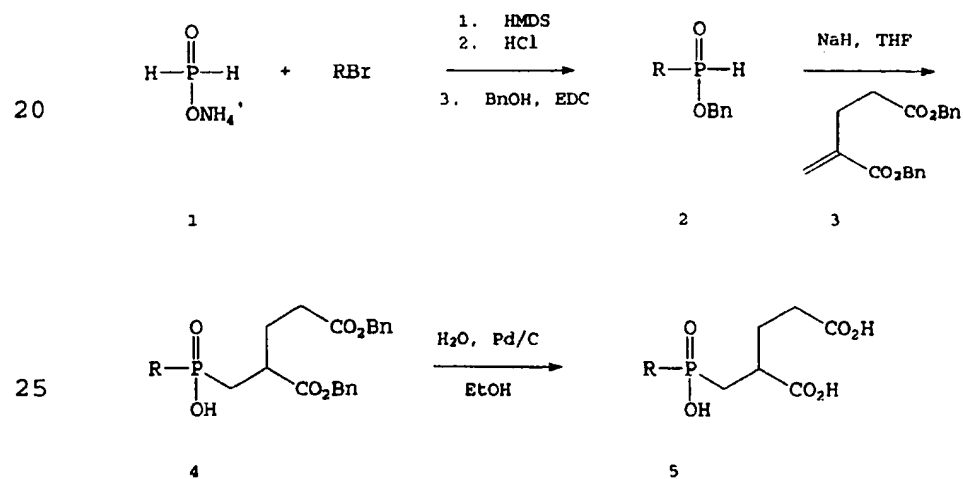
WO 98/13044

PCT/US97/14417

70

reasonable chemical substituent including without limitation the substituents listed in Scheme II and throughout the specification.

5

Scheme IV**Scheme V**

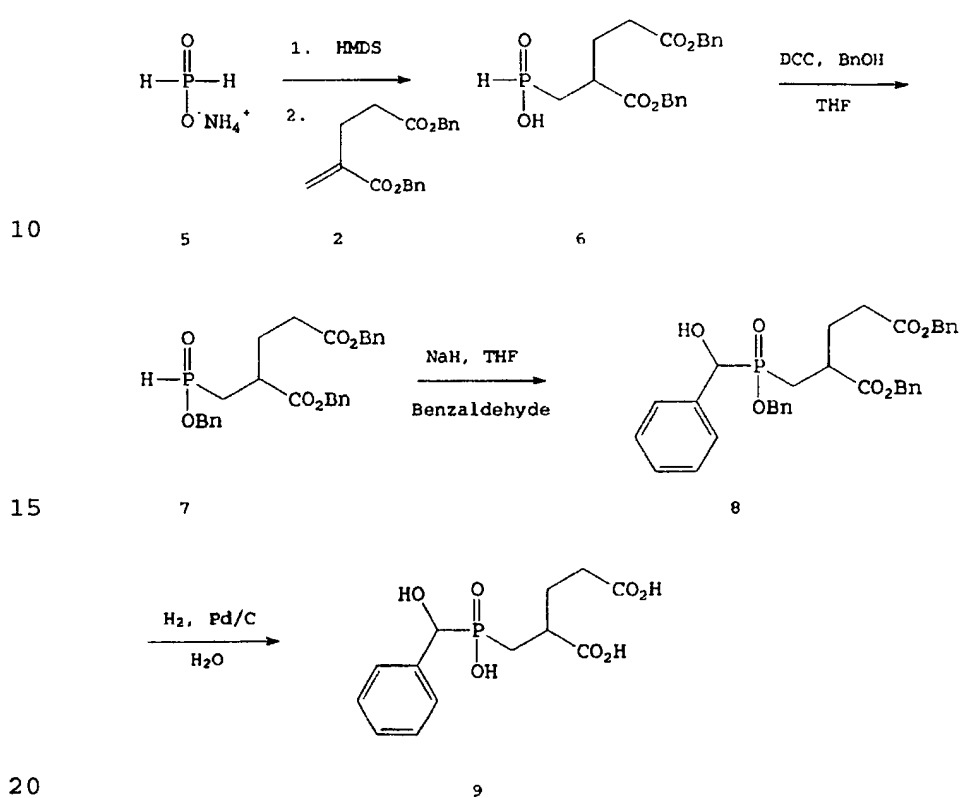
WO 98/13044

PCT/US97/14417

71

Another route for preparing the compounds of formula I allows for aromatic substitution at R_1 , and is set forth below in Scheme VI.

5

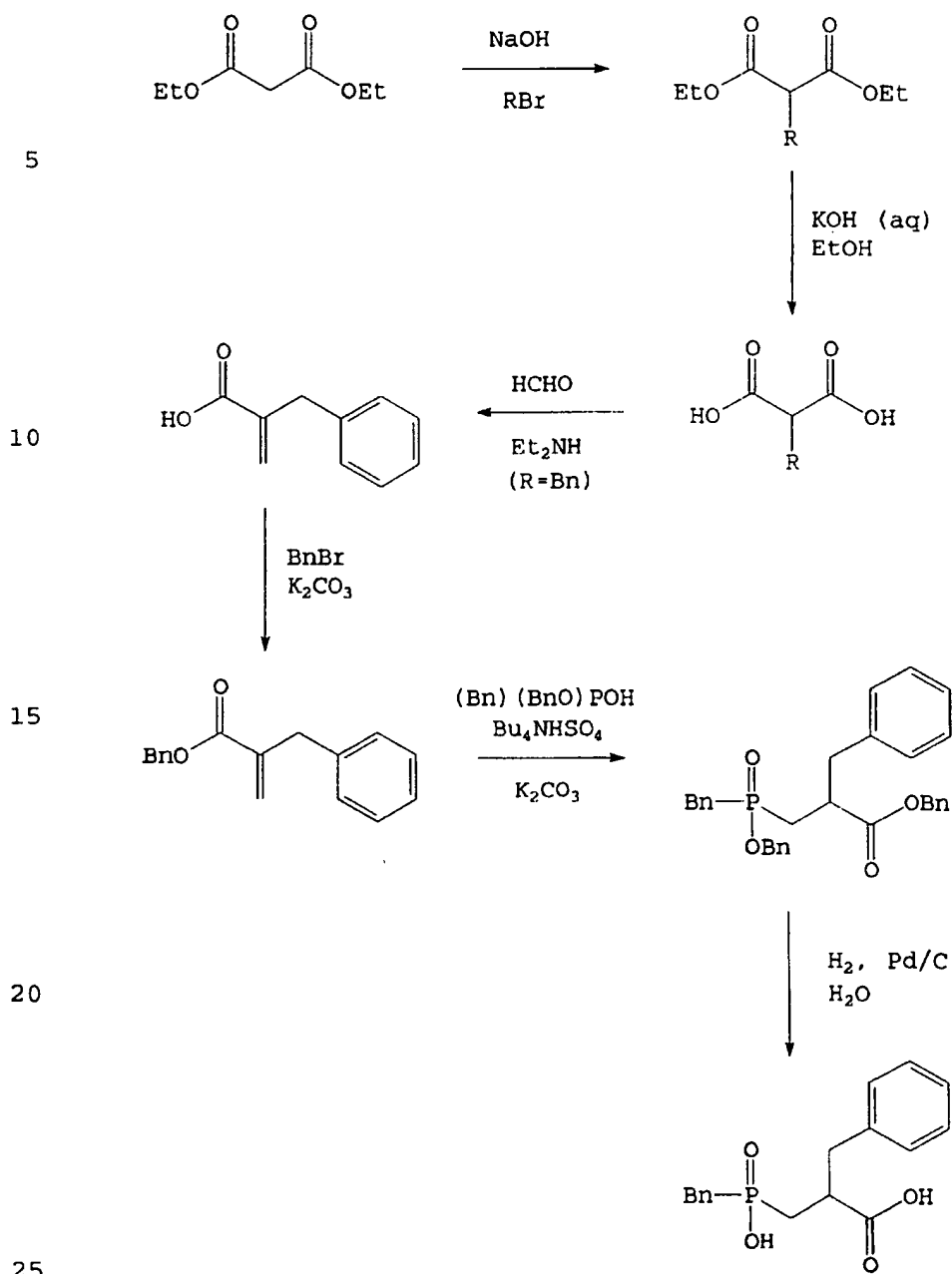
Scheme VI

Another route for preparing the compounds of formula I allows for aromatic substitution at the R_2 position, and is set forth below in Scheme VII.

WO 98/13044

PCT/US97/14417

72

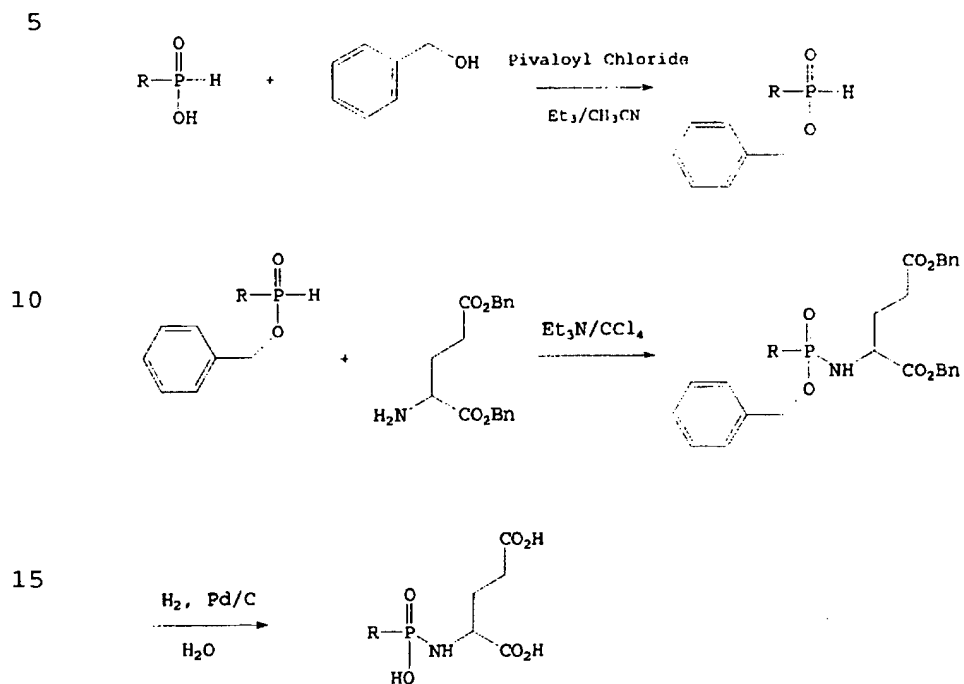
Scheme VII

WO 98/13044

PCT/US97/14417

73

Another route for preparing the compounds of formula I wherein Y is NR₅ is set forth below in Scheme VIII.

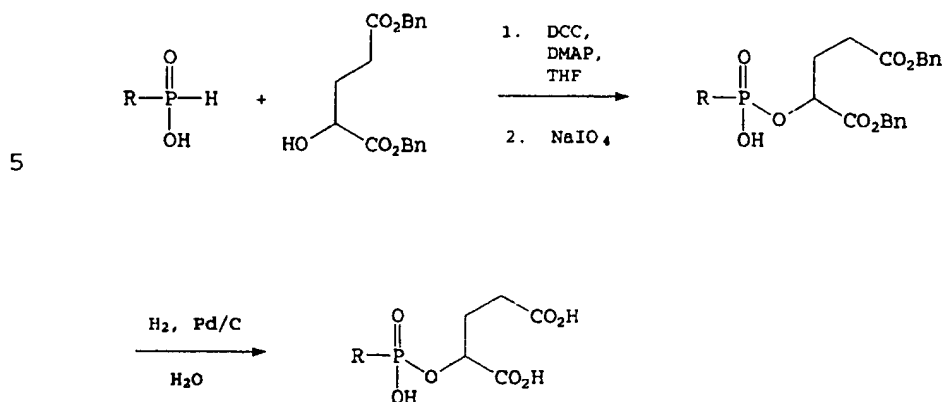
Scheme VIII

Another route for preparing the compounds of formula I wherein Y is oxygen is set forth below in Scheme IX.

WO 98/13044

PCT/US97/14417

74

Scheme IXMETHODS OF THE PRESENT INVENTION

Although not limited to any one particular theory, it is believed that NAALADase inhibitors modulate levels of glutamate by acting on a storage form of glutamate which is hypothesized to be upstream from the effects mediated by the NMDA receptor. The inventors have unexpectedly found that NAALADase inhibitors are effective in treating glutamate-related compulsive disorders.

Accordingly, the present invention further relates to a method of treating a compulsive disorder, comprising administering an effective amount of a NAALADase inhibitor to a patient in need thereof.

The compulsive disorder may be any disorder characterized by irresistible impulsive behavior.

WO 98/13044

PCT/US97/14417

75

Examples of compulsive disorders treatable by the methods of the present invention include drug dependence, eating disorders, pathological gambling, ADD and Tourette's syndrome.

5 Preferably, the compulsive disorder is drug dependence. Commonly used drugs with potential for dependence include CNS depressants (opioids, synthetic narcotics, barbiturates, glutethimide, methyprylon, ethchlorvynol, methaqualone, alcohol); anxiolytics
10 (diazepam, chlordiazepoxide, alprazolam, oxazepam, temazepam); stimulants (amphetamine, methamphetamine, cocaine); and hallucinogens (LSD, mescaline, peyote, marijuana).

 More preferably, the drug dependence is alcohol,
15 nicotine, heroin or cocaine dependence.

 Examples of NAALADase inhibitors useful for the methods of the present invention are identified above in relation to pharmaceutical compositions.

20

ROUTE OF ADMINISTRATION

 In the methods of the present invention, the compounds may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage
25 formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and

WO 98/13044

PCT/US97/14417

76

vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal or intracranial injection and infusion techniques. Invasive techniques are preferred, particularly direct administration to damaged neuronal tissue.

To be effective therapeutically as central nervous system targets, the NAALADase inhibitors used in the methods of the present invention should readily penetrate the blood-brain barrier when peripherally administered. Compounds which cannot penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The compounds may also be administered in the form of sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions. These suspensions can be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparations may also be sterile injectable solutions or suspensions in non-toxic parenterally-acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

WO 98/13044

PCT/US97/14417

77

In addition, sterile fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil such as a synthetic mono- or di-glyceride may be employed. Fatty acids such as oleic acid and its glyceride derivatives, including olive oil and castor oil, especially in their polyoxyethylated forms, are useful in the preparation of injectables. These oil solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

10 Additionally, the compounds may be administered orally in the form of capsules, tablets, aqueous suspensions or solutions. Tablets may contain carriers such as lactose and corn starch, and/or lubricating agents such as magnesium stearate. Capsules may contain
15 diluents including lactose and dried corn starch. Aqueous suspensions may contain emulsifying and suspending agents combined with the active ingredient. The oral dosage forms may further contain sweetening and/or flavoring and/or coloring agents.

20 The compounds may further be administered rectally in the form of suppositories. These compositions can be prepared by mixing the drug with suitable non-irritating excipients which are solid at room temperature, but liquid at rectal temperature such that they will melt in
25 the rectum to release the drug. Such excipients include cocoa butter, beeswax and polyethylene glycols.

WO 98/13044

PCT/US97/14417

78

Moreover, the compounds may be administered topically, especially when the conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin or the lower intestinal tract.

For topical application to the eye, or ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline or, preferably, as a solution in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, the compounds may be formulated into ointments, such as petrolatum.

For topical application to the skin, the compounds can be formulated into suitable ointments containing the compounds suspended or dissolved in, for example, mixtures with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated into suitable lotions or creams containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

WO 98/13044

PCT/US97/14417

79

Topical application to the lower intestinal tract can be effected in rectal suppository formulations (see above) or in suitable enema formulations.

The NAALADase inhibitors used in the methods of the present invention may be administered by a single dose, multiple discrete doses or continuous infusion. Since the compounds are small, easily diffusible and relatively stable, they are well suited to continuous infusion. Pump means, particularly subcutaneous pump means, are preferred for continuous infusion.

DOSAGE

Dose levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels being about 0.1 mg to about 1,000 mg. The specific dose level for any particular patient will vary depending upon a variety of factors, including the activity of the specific compound employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the rate of excretion; drug combination; the severity of the particular disease being treated; and the form of administration. Typically, in vitro dosage-effect results provide useful guidance on the proper doses for patient administration. Studies in animal models are also helpful. The considerations for

WO 98/13044

PCT/US97/14417

80

determining the proper dose levels are well known in the art.

In a preferred embodiment, the NAALADase inhibitors are administered in lyophilized form. In this case, 1 to
5 100 mg of a NAALADase inhibitor may be lyophilized in individual vials, together with a carrier and a buffer, such as mannitol and sodium phosphate. The compound may be reconstituted in the vials with bacteriostatic water before administration.

10 The NAALADase inhibitors used in the methods of the present invention may be administered in combination with one or more therapeutic agents. Specific dose levels for these agents will depend upon considerations such as those identified above for the NAALADase inhibitors.

15

ADMINISTRATION REGIMEN

For the methods of the present invention, any administration regimen regulating the timing and sequence of drug delivery can be used and repeated as necessary to
20 effect treatment. Such regimen may include pretreatment and/or co-administration with additional therapeutic agents.

To maximize protection of nervous tissue from nervous insult, the NAALADase inhibitors should be
25 administered to the affected cells as soon as possible. In situations where nervous insult is anticipated, the

WO 98/13044

PCT/US97/14417

81

compounds should be administered before the expected nervous insult. Such situations of increased likelihood of nervous insult include surgery (cartoid endarterectomy, cardiac, vascular, aortic, orthopedic);

5 endovascular procedures such as arterial catheterization (cartoid, vertebral, aortic, cardia, renal, spinal, Adamkiewicz); injections of embolic agents; coils or balloons for hemostasis; interruptions of vascularity for treatment of brain lesions; and predisposing medical

10 conditions such as crescendo transient ischemic attacks, emboli and sequential strokes. Where pretreatment for stroke or ischemia is impossible or impracticable, it is important to get the NAALADase inhibitors to the affected cells as soon as possible during or after the event. In

15 the time period between strokes, diagnosis and treatment procedures should be minimized to save the cells from further damage and death.

COMBINATION WITH OTHER TREATMENTS

20 In methods of treating nervous insult (particularly acute ischemic stroke and global ischemia caused by drowning and head trauma), the NAALADase inhibitors can be co-administered with one or more therapeutic agents, preferably agents which can reduce the risk of stroke

25 (such as aspirin), and more preferably agents which can reduce the risk of a second ischemic event (such as

WO 98/13044

PCT/US97/14417

82

ticlopidine).

The NAALADase inhibitors can be co-administered with one or more therapeutic agents either (i) together in a single formulation, or (ii) separately in individual
5 formulations designed for optimal release rates of their respective active agent. Each formulation may contain from about 0.01% to about 99.99% by weight, preferably from about 3.5% to about 60% by weight, of a NAALADase inhibitor, as well as one or more pharmaceutical
10 excipients, such as wetting, emulsifying and pH buffering agents.

In Vivo Toxicity of NAALADase Inhibitors

To examine the toxicological effect of NAALADase
15 inhibition in vivo, a group of mice were injected with 2-(phosphonomethyl)pentanedioic acid, a NAALADase inhibitor of high activity, in doses of 1, 5, 10, 30, 100, 300 and 500 mg/kg body weight. The mice were subsequently observed two times per day for 5 consecutive days. The
20 survival rate at each dose level is provided below in TABLE I. The results show that the NAALADase inhibitor is non-toxic to mice, suggesting that it would be similarly non-toxic to humans when administered at therapeutically effective amounts.

25

WO 98/13044

PCT/US97/14417

83

TABLE I

TOXICOLOGICAL EFFECTS OF NAALADASE INHIBITORS							
Dose (mg/kg)	1	5	10	30	100	300	500
5 Survival Rate After 5 days (%)	100	100	100	100	100	100	66.7

10 In Vitro Inhibition of NAALADase Activity

Various compounds of formula I were tested for in vitro inhibition of NAALADase activity. The results are provided below in Table III.

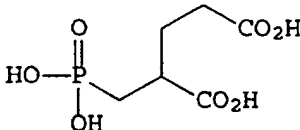
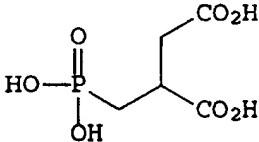
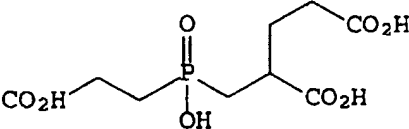
WO 98/13044

PCT/US97/14417

84

TABLE II

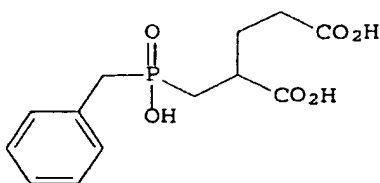
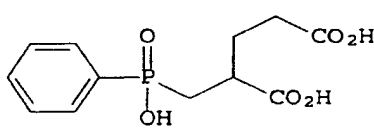
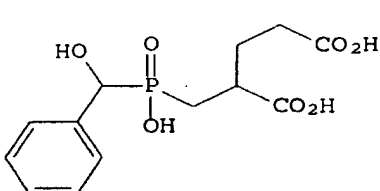
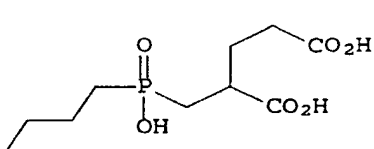
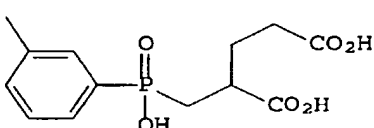
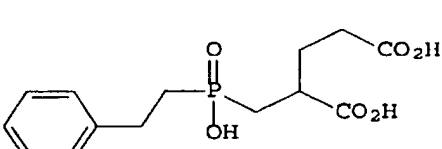
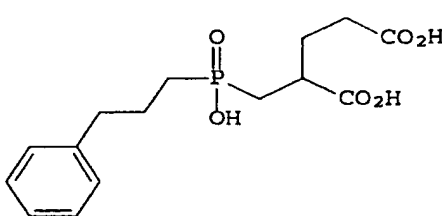
IN VITRO INHIBITION OF NAALADASE ACTIVITY

5	Compound	K_i (nM)
		0.293 ± 0.08
10	2-(phosphonomethyl)pentanedioic acid	
		700.00 ± 67.3
15	2-(phosphonomethyl)succinic acid	
		1.89 ± 0.19
20	2-[[[(2-carboxyethyl)hydroxyphosphinyl]-methyl]pentanedioic acid	

WO 98/13044

PCT/US97/14417

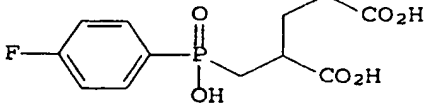
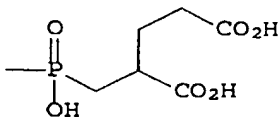
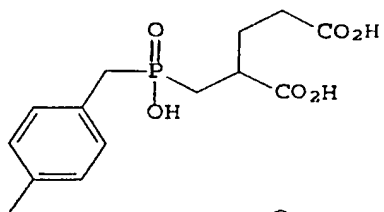
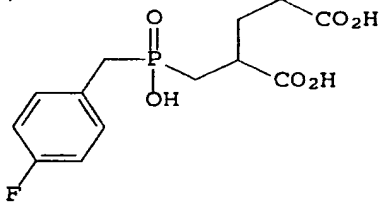
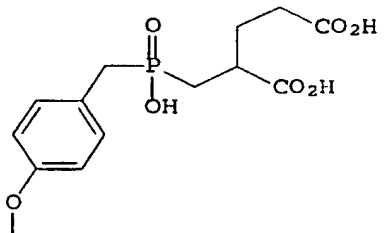
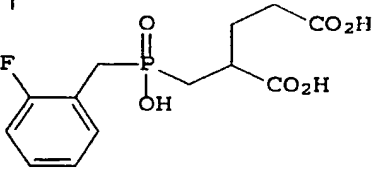
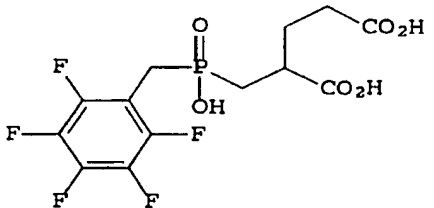
85

5		34.15
		35.85
10		54.50
15		113.50
		180.00
20		148.50
25		231.67

WO 98/13044

PCT/US97/14417

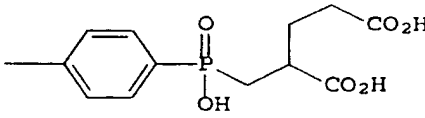
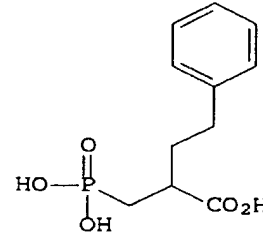
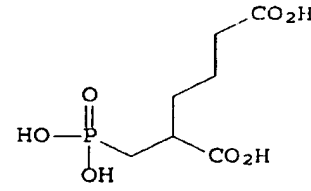
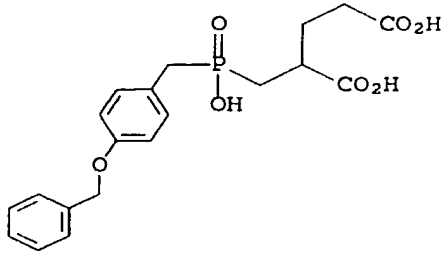
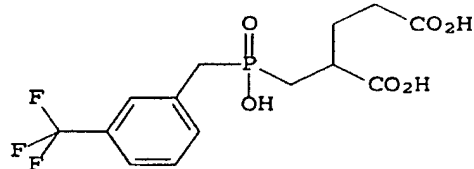
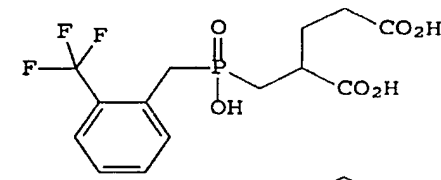
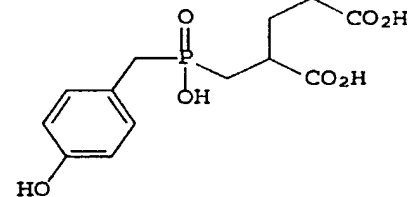
86

		532.00
5		1100.00
10		68.00
		70.00
15		89.50
20		145.00
25		22.67

WO 98/13044

PCT/US97/14417

87

		204.00
5		199.00
10		185.00
15		177.00
20		22.50
25		92.00
		117.00

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

88

The results show that 2-(phosphonomethyl)pentanedioic acid exhibits high NAALADase inhibiting activity, with a K_i of 0.293 nM. The activity of this compound is over 1000 times greater than that of previously described
5 NAALADase inhibitors.

By comparison, 2-(phosphonomethyl)succinic acid exhibits much lower NAALADase inhibiting activity, suggesting that a glutamate analog attached to the phosphonic acid contributes to its NAALADase inhibiting
10 activity.

The results also show that 2-[[[(2-carboxyethyl)-hydroxyphosphinyl]methyl]pentanedioic acid, which has an additional carboxylic acid side chain similar to the aspartate residue found in NAAG, exhibits a lower
15 NAALADase inhibiting activity than 2-(phosphonomethyl)-pentanedioic acid.

Protocol for Assaying In Vitro Inhibition of NAALADase

Activity

20 The amount of [^3H]Glu liberated from [^3H]NAAG in 50 mM Tris-Cl buffer was measured for 15 minutes at 37° C using 30-50 μg of synaptosomal protein. Substrate and product were resolved by anion-exchange liquid chromatography. Duplicate assays were performed so that
25 no more than 20% of the NAAG was digested, representing the linear range of peptidase activity. Quisqualate (100

WO 98/13044

PCT/US97/14417

89

μM) was included in parallel assay tubes to confirm the specificity of the measurements.

In Vitro Assay of NAALADase Inhibitors on Ischemia

5 To examine the *in vitro* effect of NAALADase inhibitors on ischemia, cortical cell cultures were treated with various compounds of formula I during an ischemic insult (potassium cyanide and 2-deoxyglucose) and for one hour thereafter (for experimental details,
10 see Vornov et al., *J. Neurochem*, Vol. 65, No. 4, pp. 1681-1691 (1995)).

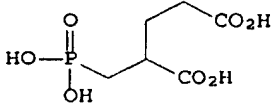
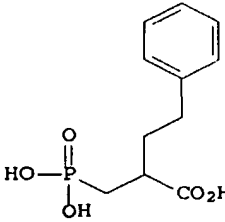
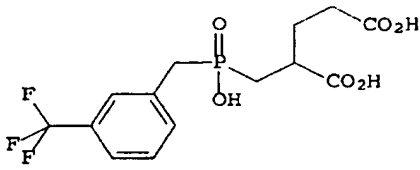
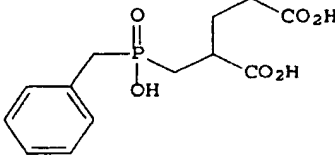
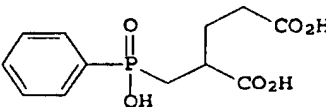
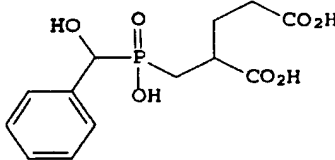
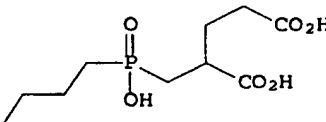
The neuroprotective effect of each tested compound is provided below in TABLE III(a). Neuroprotective effect is expressed as EC_{50} , the concentration which is
15 required to cause a 50% reduction in glutamate toxicity following an ischemic insult.

WO 98/13044

PCT/US97/14417

90

TABLE III (a)

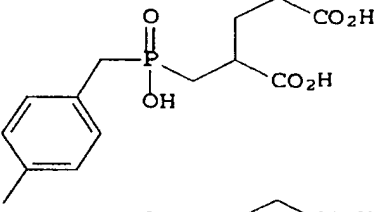
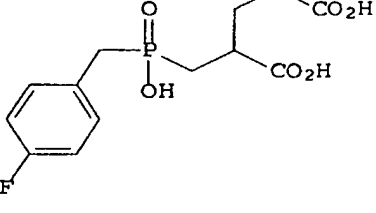
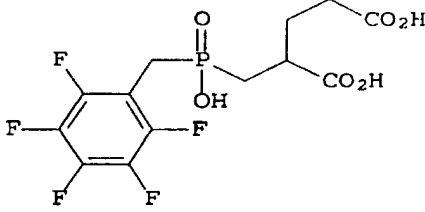
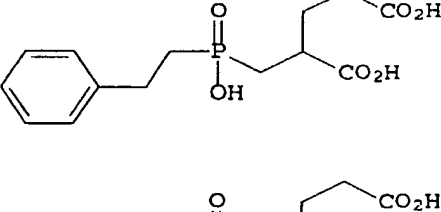
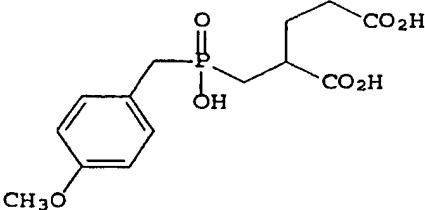
	Compound	EC ₅₀ (nM)
5		0.67
10		373.0
15		112.0
		132.0
20		100.0
		767.0
25		794.0

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

91

5		37.00
10		79.00
15		2.00
20		834.00
25		1670.00

SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

92

The dose-response of this effect, as measured by the % toxicity at different concentrations of 2-(phosphonomethyl)pentanedioic acid, is provided below in TABLE III(b) and graphically presented in FIG. 1.

5

TABLE III(b)

	<u>Dose</u>	<u>% Toxicity</u>
	Control	100.00 \pm 9.0 (n = 5)
	100 pM	66.57 \pm 4.38 (n = 5)
10	1 nM	42.31 \pm 9.34 (n = 5)
	10 nM	33.08 \pm 9.62 (n = 5)
	100 nM	30.23 \pm 9.43 (n = 5)
	1 μ M	8.56 \pm 8.22 (n = 5)

15 The results show that toxicity decreased as the concentration of 2-(phosphonomethyl)pentanedioic acid increased, suggesting that NAALADase inhibitors would be effective in treating ischemia or neuronal damage caused by ischemia.

20 The methods for this assay are described in detail below. Specifically, cell cultures were exposed to potassium cyanide and 2-deoxyglucose (2-DG) (10 mM) and analyzed for release of lactate dehydrogenase (LDH).

25

WO 98/13044

PCT/US97/14417

93

In Vitro Toxicity of NAAG

To examine the in vitro toxicity of NAAG, cortical cell cultures were treated with NAAG (in concentrations ranging from 3 μ M to 3 mM) for 20 minutes. The toxicity measurement for each concentration of NAAG is provided below in TABLE IV and graphically presented in FIG. 2.

TABLE IV

	<u>Dose of NAAG</u>	<u>% Toxicity</u>
10	3 μ M	3.51 (n = 1)
	10 μ M	4.30 \pm 3.12 (n = 3)
	30 μ M	11.40 \pm 6.17 (n = 3)
	100 μ M	12.66 \pm 5.50 (n = 3)
	300 μ M	13.50 \pm 4.0 (n = 3)
15	1 mM	21.46 \pm 4.20 (n = 3)
	3 mM	45.11 \pm 4.96 (n = 3)

The results show that toxicity increased as the concentration of NAAG increased. The toxicity is attributed to the release of glutamate by NAAG when cleaved by NAALADase.

In Vitro Assay of NAALADase Inhibitors on Toxicity of
NAAG

To examine the effect of NAALADase inhibitors on in vitro toxicity of NAAG, cortical cell cultures were

WO 98/13044

PCT/US97/14417

94

treated with 2-(phosphonomethyl)pentanedioic acid (1 μ M) during exposure to NAAG and for one hour thereafter. The toxicity measurement for each concentration of NAAG is provided below in TABLE V and graphically presented in

5 FIG. 3.

TABLE V

	<u>Dose of NAAG</u>	<u>% Toxicity</u>
	3 μ M	-4.71 (n = 1)
10	10 μ M	-3.08 \pm 0.81 (n = 3)
	30 μ M	-4.81 \pm 1.13 (n = 3)
	100 μ M	-2.87 \pm 0.78 (n = 3)
	300 μ M	-2.09 \pm 0.48 (n = 3)
	1 mM	0.26 \pm 1.11 (n = 3)
15	3 mM	16.83 \pm 8.76 (n = 3)

When compared to the results of FIG.2/TABLE IV, the results of FIG.3/TABLE V show that toxicity decreased considerably after treatment with the NAALADase inhibitor, suggesting that it would be effective in

20 treating glutamate abnormalities.

In Vitro Assay of NAALADASE Inhibitors on Ischemia at
Different Times of Administration

25 To examine the effect of NAALADase inhibitors on in vitro ischemic toxicity at different times of

WO 98/13044

PCT/US97/14417

95

administration, cortical cell cultures were treated with 2-(phosphonomethyl)pentanedioic acid (i) during an ischemic insult and for one hour thereafter (exposure and recovery); (ii) for one hour following ischemic insult (recovery only); and (iii) for one hour beginning 30 minutes after ischemic insult (delayed 30 minutes). The toxicity measurement for each time of administration is provided below in TABLE VI and graphically presented in FIG. 4.

10

TABLE VI

	<u>Time of Administration Relative to Ischemic Insult</u>	<u>% Toxicity</u>
	Control	100.00%
15	Exposure & Recovery	2.54%
	Recovery Only	9.03%
	Delayed 30 Minutes	31.49%

The results show that significant neuronal protection is achieved when NAALADase inhibitors are administered during exposure and recovery from an ischemic insult, and even after a 30 minute delay following the ischemic insult.

25

WO 98/13044

PCT/US97/14417

96

Protocol for In Vitro Toxicity Assaya. Cell Culture

Dissociated cortical cell cultures are prepared using the papain-dissociation method of Heuttner and
5 Baughman (1986) as modified by Murphy and Baraban (1990). See TABLE VII for the Dissociated Culture Protocol as used herein. Fetuses of embryonic day 17 are removed from timed pregnancy rats (Harlan Sprague Dawley). The cortex is rapidly dissected out in Dulbecco's phosphate-
10 buffered saline, stripped of meninges, and incubated in a papain solution for 15 minutes at 37° C. The tissue is then mechanically triturated and pelleted at 500 g (1000-2000 rpm on swinging bucket Beckman). The pellet is resuspended in a DNAase solution, triturated with a 10 ml
15 pipette x15-20, layered over a "10 x 10" solution containing albumin and trypsin inhibitor (see TABLE VII for an example of a "10 x 10" solution), repelleted, and resuspended in a plating medium containing 10% fetal bovine serum (HyClone A-1111-L), 5% heat-inactivated
20 Equine serum (HyClone A-3311-L), and 84% modified Earle's basal medium (MEM) (Gibco 51200-020) with high glucose (4.5 g/L), and 1 g/L NaHCO₃. Each 24-well plate is pretreated with poly-D-lysine (0.5 ml/well of 10 µg/ml) for 1 h and rinsed with water before plating. Cultures
25 are plated at 2.5 x 10⁶ cells/ml with each well of a 24 well plate receiving 500µl/well. Alternatively, 35 mm

WO 98/13044

PCT/US97/14417

97

dishes can be plated at 2 ml/dish, 6 well plates at 2 ml/well, or 12 well plates at 1 ml/well. After plating, 50% of the medium is changed every 3-4 days with growth serum containing 5% heat-inactivated Equine serum (HyClone A-3311-L), 95% modified Earle's basal medium (MEM) (Gibco 51200-020), and 1% L-Glutamine (Gibco 25030-081). Experiments are performed after 21 days in cultures. Cultures are maintained in a 5% CO₂ atmosphere at 37° C. These methodologies are described in further detail below in the TABLE VII.

WO 98/13044

PCT/US97/14417

98

TABLE VII

DISSOCIATED CULTURE PROTOCOL	
I. PREPARE SOLUTIONS	
Stocks/Solutions	
<p>5 <u>DNAase Stock, 1 ml</u> (100x) 5 mg DNAase I (Worthington LS002004); 1 ml dissoc. EBSS; 10 freeze as 50 μl aliquots.</p>	<p><u>Dulbecco's PBS, 500 ml</u> 4 gm NaCl (J.T. Baker 3624-01); 1.06 gm $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (Fisher S373-3); 100 mg KCl (Fisher P217-500); 100 mg KH_2PO_4 (Sigma P-0662); 500 ml dH_2O; adjust pH to 7.4 and sterile filter.</p>
<p>15 <u>Dissociated EBSS, 500 ml</u> 1.1 gm NaHCO_3; 50 ml EBSS stock (Gibco 14050-025); 450 ml dH_2O; sterile filter.</p>	<p><u>EDTA Stock, 10 ml</u> 184.2 mg EDTA sodium salt (Sigma ED4S); 10 ml dH_2O; sterile filter.</p>

WO 98/13044

PCT/US97/14417

99

DISSOCIATED CULTURE PROTOCOL			
5	<table><tr><td><u>10 and 10 Stock, 10 ml</u> 100 mg BSA (Sigma A-4919); 100 mg Trypsin Inhibitor from Egg White (Sigma T-2011); 10 ml dissoc. EBSS; sterile filter.</td><td><u>Poly-D-Lysine Stock, 5 ml</u> 5 mg Poly-D-Lysine, 100-150 K (Sigma P-6407); 5 ml sterile water; keep frozen.</td></tr></table>	<u>10 and 10 Stock, 10 ml</u> 100 mg BSA (Sigma A-4919); 100 mg Trypsin Inhibitor from Egg White (Sigma T-2011); 10 ml dissoc. EBSS; sterile filter.	<u>Poly-D-Lysine Stock, 5 ml</u> 5 mg Poly-D-Lysine, 100-150 K (Sigma P-6407); 5 ml sterile water; keep frozen.
<u>10 and 10 Stock, 10 ml</u> 100 mg BSA (Sigma A-4919); 100 mg Trypsin Inhibitor from Egg White (Sigma T-2011); 10 ml dissoc. EBSS; sterile filter.	<u>Poly-D-Lysine Stock, 5 ml</u> 5 mg Poly-D-Lysine, 100-150 K (Sigma P-6407); 5 ml sterile water; keep frozen.		
10	<table><tr><td>Media</td><td></td></tr></table>	Media	
Media			

WO 98/13044

PCT/US97/14417

100

DISSOCIATED CULTURE PROTOCOL	
<p><u>Dissociated growth, 500 ml</u></p> <p>500 ml MEM (Gibco 51200-020) containing glucose and NaHCO₃, (2.25 gm glucose and 0.5 gm NaHCO₃);</p> <p>25 ml heat-inactivated Equine Serum (HyClone A-3311-L);</p> <p>5 ml L-Glutamine (200 mM, 100x stock, Gibco 25030-081);</p> <p>sterile filter.</p> <p>15 ml heat-inactivated Equine Serum (HyClone A-3311-L);</p> <p>3 ml L-Glutamine (200 mM, 100x stock, Gibco 25030-081); (Gibco 15140-015);</p> <p>1 ml Penicillin-Streptomycin stock.</p>	<p><u>Plating media, 300 ml</u></p> <p>250 ml MEM containing glucose and sodium bicarbonate (2.25 gm glucose and 0.5 gm NaHCO₃ in 500 ml Gibco MEM 51200-020);</p> <p>30 ml Fetal Bovine Serum (HyClone A-1111-L).</p>

WO 98/13044

PCT/US97/14417

101

DISSOCIATED CULTURE PROTOCOL	
For papain dissociation:	For DNAase treatment:
4 mg Cysteine (C-8277); 25 ml dissoc. EBSS; 250 μ l Papain stock (Worthington LS003126); place in 37°C waterbath until clear.	<u>DNAase, 5 ml</u> 4.5 ml dissoc. EBSS; 500 μ l "10 and 10" stock; 50 μ l DNAase stock. <u>"10 and 10", 5 ml</u> 4.5 ml of EBSS; 500 μ l "10 and 10" stock.
II. COAT DISHES	
Use poly-d-lysine stock at 1:100 dilution to coat 24-well plates (0.5 ml/well) or at 1:10 dilution to coat 35 mm glass cover slips (1.0 ml/cover slip). Leave until end of dissection.	

WO 98/13044

PCT/US97/14417

102

DISSOCIATED CULTURE PROTOCOL**III. DISSECT TISSUE**

Use Harlan Sprague-Dawley timed pregnancy rats,
5 ordered to arrive at E-17.

Decapitate, spray abdomen down with 70% EtOH.

Remove uterus through midline incision and place in
sterile dPBS.

Remove brains from embryos, leaving them in dPBS.

10 Brain removal: Penetrate skull and skin with fine
forceps at lambda. Pull back to open posterior fossa.
Then move forceps anteriorly to separate sagittal
suture. Brain can be removed by scooping back from
15 olfactory bulbs under the brain.

Move brains to fresh dPBS; subsequently, dissect away
from cortex.

WO 98/13044

PCT/US97/14417

103

DISSOCIATED CULTURE PROTOCOL**IV. PAPAIN DISSOCIATION**

Transfer cortices equally to two 15 ml tubes
containing sterile papain solution, maintained at 37°
C.

Triturate xl with sterile 10 ml pipette.

Incubate only for 15 minutes at 37° C.

Spin at 500 G for 5 minutes (1000-2000 RPM on swinging
bucket Beckman).

V. DNAase TREATMENT

Remove supernatant and any DNA gel layer from cell
pellet (or pick up and remove pellet with pipette).

Move cell pellet to DNAase solution.

Triturate with 10 ml pipette, x15-20.

Layer cell suspension over the "10 and 10" solution by
pipetting it against the side of the tubes.

Spin again at 500 G for 5 minutes (cells will spin
into "10 and 10" layer).

Wash tube sides with plating media without disturbing
pellet.

Pipette off the media wash and repeat the wash.

WO 98/13044

PCT/US97/14417

104

DISSOCIATED CULTURE PROTOCOL

VI. PLATE

Add about 4.5 ml plating media to each pellet for 5 ml volume.

5 Re-suspend with 10 ml pipette.

Pool cells into a single tube.

Quickly add 10 μ l of the suspended cells to a hemocytometer so that they do not settle.

10 Count cells per large square, corresponding to 10 million cells/ml.

Put re-suspended cells into a larger container so that they number 2.5 million cells/ml.

Triturate to homogeneity.

15 Finish coating plates:

Aspirate or dump Lysine;

Wash x1 with sterile water and dump.

20 Add plating media, with cells, to the plates as follows:

35 mm dishes 2 ml/dish;

6 well plate 2 ml/well;

12 well plate 1 ml/well;

24 well plate 500 μ l/well.

WO 98/13044

PCT/US97/14417

105

DISSOCIATED CULTURE PROTOCOL	
VII. FEED	
Cultures are usually made on Thursdays. Start feeding twice a week; beginning the following Monday, feedings on Mondays and Fridays. 5 Remove 50% of volume and replace with fresh growth media.	

b. Ischemic Insult using potassium cyanide and

10 **2-deoxyglucose**

Twenty-one to twenty-four days following the initial cortical cell plating, the experiment is performed. The cultures are washed three times in HEPES buffered saline solution containing no phosphate. The cultures are then
15 exposed to potassium cyanide (KCN) (5 mM) and 2-deoxyglucose (2-DG) (10 mM) for 20 minutes at 37° C. These concentrations were shown previously to induce maximal toxicity (Vornov et al., *J. Neurochem*, Vol. 65, No. 4, pp. 1681-1691 (1995)). At the end of 24 hours,
20 the cultures are analyzed for release of the cytosolic enzyme lactate dehydrogenase (LDH), a standard measure of cell lysis. LDH measurements are performed according to the method of Koh and Choi, *J. Neuroscience Methods* (1987).

WO 98/13044

PCT/US97/14417

106

c. NAAG Induced Neurotoxicity

Cultures are assessed microscopically and those with uniform neuronal densities are used in the NAAG neurotoxicity trials.

5 At the time of the experiment, the cultures are washed once in HEPES-buffered saline solution (HBSS; NaCl 143.4 mM, HEPES 5 mM, KCl 5.4 mM, MgSO₄ 1.2 mM, NaH₂PO₄ 1.2 mM, CaCl₂ 2.0 mM, D-glucose 10 mM) (Vornov et al., 1995) and then exposed to various concentrations of NAAG
10 for 20 minutes at 37° C. NAAG concentrations range from 3 μM to 3 mM, and include 3 μM, 10 μM, 30 μM, 100 μM, 300 μM, 1 mM, and 3 mM. At the end of exposure, the cells are washed once with HEPES buffered saline solution and then replaced with serum free modified Earle's basal
15 medium. The cultures are then returned to the CO₂ incubator for 24 hour recovery.

d. Lactate Dehydrogenase Assay

Release of the cytosolic enzyme lactate dehydrogenase (LDH), a standard measure of cell lysis, is
20 used to quantify injury (Koh and Choi, 1987). LDH-activity measurements are normalized to control for variability between culture preparations (Koh and Choi, 1987). Each independent experiment contains a control condition in which no NAALADase inhibitors are added; a
25 small amount of LDH activity is found in these controls. This control measurement is subtracted from each

WO 98/13044

PCT/US97/14417

107

experimental point. These values are normalized within each experiment as a percentage of the injury caused by NAAG/ischemia. Only main effects of NAALADase inhibitors are considered; interactions between dose and condition are not examined statistically.

A measurement of the potency of each compound tested is made by measuring the percentage of LDH release into the growth media after exposure to NAAG/ischemia plus NAALADase inhibitor or NAAG/ischemia plus saline (control). Since high concentrations of glutamate may be toxic to cells in certain circumstances, measurement of glutamate toxicity is observed using LDH as a standard measurement technique.

In Vivo Assay of NAALADase Inhibitors on Cortical Injury following MCAO in SHRSP Rats

To examine the effect of NAALADase inhibitors on cortical injury *in vivo*, the infarct volume was measured in SHRSP rats which had sustained middle cerebral artery occlusion (MCAO) and had subsequently been treated with (i) saline; (ii) 10 mg/kg of 2-(phosphonomethyl)-pentanedioic acid followed by 2 mg/kg/hr of 2-(phosphonomethyl)pentanedioic acid for 1 hour; or (iii) 100 mg/kg of 2-(phosphonomethyl)pentanedioic acid followed by 20 mg/kg/hr of 2-(phosphonomethyl)-pentanedioic acid for one hour.

WO 98/13044

PCT/US97/14417

108

The cortical injury volume for each group of rats is provided below in TABLE VIII and graphically presented in FIG. 5.

5

TABLE VIII

10

15

Cortical Injury Volume (mm ³)±S.E.M.	
Control	184.62±33.52 (n = 10)
10 mg/kg	135.00±32.18 (n = 10)
100 mg/kg	65.23±32.18 (n = 10)
Cortical Injury Volume (% injury)±S.E.M.	
Control	34.44±6.53 (n = 10)
10 mg/kg ³	29.14±7.68 (n = 10)
100 mg/kg	13.98±6.64 (n = 10)
Cortical Protection (% protection)	
Control	0%
10 mg/kg	27%
100 mg/kg	65%

The results show that cortical injury volume decreased and cortical protection increased as the amount of NAALADase inhibitor increased, further supporting the neuroprotective effect of the NAALADase inhibitor.

WO 98/13044

PCT/US97/14417

109

Protocol for In Vivo Assay of NAALADase Inhibitors on
Cortical Injury

A colony of SHRSP rats is bred at Johns Hopkins School of Medicine from three pairs of male and female rats obtained from the National Institutes of Health (Laboratory, Sciences Section, Veterinary Resources Program, National Center for Research Resources, Bethesda, MD). All rats are kept in a virus-free environment and maintained on regular diet (NIH 31, Zeigler Bros, Inc.) with water ad libitum. All groups of rats are allowed to eat and drink water until the morning of the experiment.

Transient occlusion of the middle cerebral artery (MCA) is induced by advancing a 4-0 surgical nylon suture into the internal carotid artery (ICA) to block the origin of the MCA (Koizumi, 1986; Longa, 1989; Chen, 1992). The rats are anesthetized with 4% halothane, and maintained with 1.0% to 1.5% halothane in air enriched oxygen using a face mask. Rectal temperature is maintained at $37.0 \pm 0.5^{\circ}\text{C}$ throughout the surgical procedure using a heating lamp. The right femoral artery is cannulated for measuring blood gases (pH, oxygen tension $[\text{PO}_2]$, carbon dioxide tension $[\text{PCO}_2]$) before and during ischemia, for monitoring blood pressure during the surgery. The right common carotid artery (CCA) is exposed through a midline incision; a self-retraining

WO 98/13044

PCT/US97/14417

110

retractor is positioned between the digastric and mastoid muscles, and the omohyoid muscle is divided. The right external carotid artery (ECA) is dissected and ligated. The occipital artery branch of the ECA is then isolated and coagulated. Next, the right internal carotid artery (ICA) is isolated until the pterygopalatine artery is exposed, and carefully separated from the adjacent vagus nerve. The pterygopalatine artery is ligated with 4-0 silk suture close to its origin.

After the CCA is ligated with 4-0 silk suture, a 4-0 silk suture to prevent bleeding from a puncture site, through which a 2.5 cm length of 4-0 monofilament nylon suture (Ethilon), its tip rounded by heating near a electric cautery, is introduced into the ICA lumen. A 6-0 silk suture is tightened around the intraluminal nylon suture at the bifurcation to prevent bleeding, and the stretched sutures at the CCA and the ICA are released. The nylon suture is then gently advanced as far as 20 mm.

Anesthesia is terminated after 10 minutes of MCA occlusion in both groups, and the rats were awakened 5 minutes thereafter. After 2 hours of ischemia, anesthesia is reanesthetized, and reperfusion is performed by withdrawing the intraluminal nylon suture until the distal tip became visible at the origin of the ICA.

Arterial pH and PaCO₂, and partial pressure of

WO 98/13044

PCT/US97/14417

111

oxygen (PaO_2) are measured with a self-calibrating Radiometer electrode system (ABL 3; Copenhagen, Denmark). Hemoglobin and arterial oxygen content are measured with a hemoximeter (Radiometer, Model OSM3; Copenhagen, Denmark). Blood glucose is measured with a glucose analyzer (model 2300A, Yellow Springs Instruments, Yellow Springs, OH).

Each group is exposed to 2 hours of right MCA occlusion and 22 hours of reperfusion. All variables but the rectal temperature are measured at baseline, at 15 minutes and 45 minutes of right MCA occlusion. The rectal temperature is measured at baseline, at 0 and 15 min of MCA occlusion, and at 0 and 22 hours of reperfusion.

15

In Vivo Assay of NAALADase Inhibitors on Brain Injury following MCAO in Sprague-Dawley Rats

To examine the neuroprotective effect of NAALADase inhibitors on brain injury *in vivo*, Sprague-Dawley rats were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid before and after sustaining a 2 hour transient middle cerebral artery occlusion (MCAO). In the control group ($n = 8$), the rats received an IP injection of saline 30 minutes post-occlusion followed by IV saline infusion at a rate of 0.5 ml/hr. In the drug treated groups, the rats received an

WO 98/13044

PCT/US97/14417

112

IP injection of 2-(phosphonomethyl)pentane-dioic acid at a dose of 100 mg/kg at 20 minutes pre-occlusion (n = 5), 30 minutes post-occlusion (n = 9), 60 minutes post-occlusion (n = 7), or 120 minutes post-occlusion (n = 4), followed by a 20 mg/kg/hr IV infusion for 4 hours (infusion rate = 0.5 ml/hr). There was a 15 minute delay between IP and IV treatments for each rat. Twenty two hours following the reperfusion, the rats were euthanized and their brains were removed. Seven coronal sections (2 mm thick) were taken and stained with 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 20 minutes and then fixed in 10% formalin. The anterior and posterior surface of the most rostral brain section and the posterior surface of each of the other 6 sections were imaged. The quantification of infarct size of each brain was obtained using a computer aided-digital imaging analysis system (LOATS). The brain regions completely lacking TTC-staining were characterized as representative of infarcted tissue. The total infarct volume for each rat was calculated by numeric integration of the respective sequential brain areas.

The total infarct volume for each group of rats is graphically presented in FIG. 6.

Vehicle treated rats exhibited a mean total brain infarct volume of $293 \pm 26 \text{ mm}^3$. Rats treated with 2-(phosphonomethyl)pentanedioic acid either before or after

WO 98/13044

PCT/US97/14417

113

the ischemic insult exhibited significantly lower mean total brain infarct volumes of $122 \pm 26 \text{ mm}^3$ ($p = 0.003$ vs. vehicle) for 20 minute pre-treatment, $208 \pm 40 \text{ mm}^3$ ($p = 0.2$ vs. vehicle) for 30 minute post-treatment, 125 ± 57 5 mm^3 ($p = 0.015$ vs. vehicle) for 60 minute post-treatment, and $133 \pm 35 \text{ mm}^3$ ($p = 0.005$ vs. vehicle) for 120 minute post-treatment. These results indicate that 2-(phosphonomethyl)pentanedioic acid is neuroprotective in rat MCAO model of stroke when administered up to 2 hours 10 post-occlusion.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Brain Injury

Male Sprague-Dawley rats (260-320 g) were used. 15 Prior to the experiment, the rats were individually housed and allowed free access to food and water. Each rat received two surgeries: jugular vein cannulation for IV infusion and MCAO. During surgeries, the rat was anesthetized with 2% halothane delivered in oxygen via an 20 inhalation mask. The body temperature was monitored and regulated at normothermic level using a homeothermic heating system. First, a PE-50 polyethylene catheter was inserted into the right jugular vein. One hour later, the rat was reanesthetized for MCAO surgery. The MCAO 25 was achieved using the endovascular suture method described by Long et al., Stroke, Vol. 20, pp. 84-91

WO 98/13044

PCT/US97/14417

114

(1989). Specifically, the right external carotid artery (ECA) was exposed, coagulated and transected. A 3-0 monofilament nylon suture with a blunted tip was introduced into the proximal stump of the ECA via an arteriotomy and advanced 20 mm from the carotid bifurcation until it lodged in the proximal region of the anterior cerebral artery, thereby occluding the origin of the MCA. The rats were allowed to wake up; 2 hours later, the rats were reanesthetized for reperfusion, during which the nylon suture was retracted to the stump of the ECA allowing blood recirculation to the MCA.

In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels

To examine the effect of NAALADase inhibitors on hyperglutamatergic disorders *in vivo*, rats with stroke-induced rise in brain glutamate levels were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIGS. 7, 8 and 9.

The results show that 2-(phosphonomethyl)-pentanedioic acid treatment (100 mg/kg IP followed by 20 mg/kg/hr IV) significantly attenuated stroke-induced extracellular glutamate increases in the striatum (FIG. 7) as compared to vehicle treated rats ($p < 0.05$), and completely prevented concurrent glutamate changes in the

WO 98/13044

PCT/US97/14417

115

parietal cortex ($p < 0.01$; FIG. 8). In contrast, there was no significant effect of the stroke itself on glutamate in the frontal cortex and no subsequent difference between the vehicle and 2-(phosphonomethyl)-
5 pentanedioic acid treated groups (FIG. 9). Values are expressed as % baseline where baseline constitutes the mean of three consecutive 20 minute samples preceding stroke. Absolute basal (pretreatment) values for glutamate (mean \pm SEM) in caudate, parietal and frontal
10 cortices were 0.25 ± 0.1 , 1.1 ± 0.3 and 0.6 ± 0.1 μ M, respectively, in the vehicle treated rats, and 0.46 ± 0.1 , 2.0 ± 0.7 and 0.9 ± 0.3 μ M, respectively, in the 2-(phosphonomethyl)pentanedioic acid treated rats.

15 **Protocol for In Vivo Assay of NAALADase Inhibitors on Stroke-Induced Rise in Brain Glutamate Levels**

Male Sprague Dawley rats (270-330 g, $n = 5-6$ per group) were implanted with concentric microdialysis probes similar to previously described procedures
20 (Britton et al., *J. Neurochem.*, Vol. 67, pp. 324-329 (1996)). In brief, under halothane anaesthesia, probes (constructed in-house using Cuprophane capillary membrane; 10K mw cut off; 2 mm dialyzing length) were implanted into the frontal cortex (AP = +3.5; ML = 3; DIV
25 = 3), caudate nucleus (AP = 0; ML = 3; DV = 6.6), and parietal cortex (AP = -2; ML = 5; DV = 3) (coordinates in

WO 98/13044

PCT/US97/14417

116

mm relative to bregma and dura, respectively), regions believed to represent core and penumbral areas of ischemia-induced injury. Glutamate levels in dialysate were determined using precolumn o-phthaldialdehyde derivatization, followed by HPLC with fluorometric detection.

Approximately 20 hours after probe implantation, the rats were dialyzed with perfusion fluid (125 mM NaCl, 2.5 mM KCl, 1.18 mM MgCl₂, and 1.26 mM CaCl₂) at a rate of 2.5 μ l/min. Following a 60 minute stabilization period, dialysis samples were collected every 20 minutes. After collecting 3 baseline samples, the rats were anaesthetized with halothane and subjected to temporary ischemia using the filament method of MCAO (Britton et al., *Life Sciences*, Vol. 60, No. 20, pp. 1729-1740 (1997)). In brief, the right external carotid artery (ECA) was exposed and its branches coagulated. A 3-0 monofilament nylon suture was introduced into the internal carotid artery via an arteriotomy in the ECA and advanced until it lodged in the proximal region of the anterior cerebral artery, thus occluding the origin of the MCA. The endovascular suture was retracted to allow reperfusion 2 hours after occlusion.

Body temperature was maintained normothermic throughout stroke surgery and reperfusion procedures. The rats were dosed IP with 100 mg/kg 2-

WO 98/13044

PCT/US97/14417

117

(phosphonomethyl)pentanedioic acid at -20 minute pre-occlusion and IV with 20 mg/kg/hr for 4 hours at the time of occlusion. Dialysis samples were collected every 20 minutes from unanesthetized rats. Following 24 hours of reperfusion, the rats were sacrificed, their brains were removed, and 7 coronal sections (2 mm thick) were taken from the region beginning 1 mm from the frontal pole and ending just rostral to the cortico-cerebellar junction. Analysis of ischemic cerebral damage was achieved using TTC staining and computer assisted image analysis as described by Britton et al. (1997), *supra*.

**In Vivo Assay of NAALADase Inhibitors on Myelin
Formation Following Sciatic Nerve Cryolesion**

It was recently demonstrated that NAALADase is down-regulated in glial cells as they start to form myelin and is absent in myelinating Schwann cells. Based on this data, the inventors hypothesized that inhibition of NAALADase may affect the signaling mechanism between axons and Schwann cells and result in increasing myelination. To test this hypothesis, the inventors examined the effect of 2-(phosphonomethyl)pentanedioic acid on nerve regeneration and myelination following cryolesion of the sciatic nerve in male mice.

The results are provided below in TABLE IX and graphically presented in FIG. 10(a) and FIG. 10(b).

WO 98/13044

PCT/US97/14417

118

TABLE IX

IN VIVO EFFECT OF NAALADASE INHIBITORS ON MYELIN
FORMATION FOLLOWING SCIATIC NERVE CRYOLESION

	2- (phosphonomethyl) - pentanedioic acid	vehicle
5		
	ratio of # of myelinated axons (drug/vehicle)	1.5
10		
	# of myelinated lamellae (ave. + SEM)	16.53 ± 0.65 13.77 ± 0.09
15		
	% increase of myelinated lamellae over vehicle	20%
20	significance by t-test	p < 0.005

As detailed in FIG. 10(a) and FIG. 10(b), both light
and transmission electron microscopy (TEM) examination of
the nerve 3 mm distal to the site of cryolesion

WO 98/13044

PCT/US97/14417

119

demonstrated a significant increase in the number of myelinated axons (1.5-fold increase) and myelin thickness (20% increase, $p < 0.005$), as compared to nerves in mice treated with vehicle.

5 FIG. 10(a) and FIG. 10(b) show a photomicrograph of this effect. Sections were stained with toluidine blue which stains myelin. Sciatic nerves treated with 2-(phosphonomethyl)-pentanedioic acid containing implants, compared with sciatic nerves treated with vehicle
10 containing implants, exhibited an increase in myelinated axon number as well as an increase in myelin thickness.

Protocol for In Vivo Assay of NAALADase Inhibitors on Myelin Formation Following Sciatic Nerve Cryolesion

15 Cryolesion of the mouse sciatic nerve was performed according to Koenig et al., *Science*, Vol. 268, pp. 1500-1503 (June 1995). In brief, each mouse was anesthetized and its sciatic nerve was exposed in the upper thigh and cryolesioned using a copper cryode (diameter = 0.5 mm)
20 that was dipped in liquid nitrogen and repeatedly applied to the upper part of the nerve. The extent of the lesion was approximately 1 mm.

2- (Phosphonomethyl)pentanedioic acid was incorporated into silicone strips according to the method
25 of Connold et al., *Developmental Brain Res*, Vol. 28, pp. 99-104 (1986), and was implanted at the site of

WO 98/13044

PCT/US97/14417

120

cryolesion on day 0 and replaced on days 3, 6, 9 and 12. Approximately 2.5 μ g/day of 2-(phosphonomethyl)-pentanedioic acid was released from the silicone implants each day. Both right and left sciatic nerves of each mouse were lesioned; right-side nerves were treated with silicone implant strips containing vehicle alone while left-side nerves were treated with silicone implants containing 2-(phosphonomethyl)pentanedioic acid. Fifteen days after surgery, the mice were sacrificed and their sciatic nerve segments were collected and processed for light microscopy and TEM analysis. Randomly chosen fields 2-3 mm distal to the lesion were qualitatively analyzed by light microscopy using 1-micrometer-thick toluidine blue stained cross sections and photographic images were captured.

In Vivo Assay of NAALADase Inhibitors on Parkinson's Disease

To examine the effect of NAALADase inhibitors on Parkinson's Disease in vivo, MPTP lesioned mice were treated with 2-(phosphonomethyl)pentanedioic acid or a vehicle.

The percent of dopaminergic neurons for each group of mice is provided below in TABLE X and graphically presented in FIG. 11.

WO 98/13044

PCT/US97/14417

121

TABLE X

IN VIVO EFFECT OF NAALADASE INHIBITORS ON PARKINSON'S
DISEASE

5		Percent Strial TH Innervation Density (mean \pm SEM)
10	vehicle/vehicle	24.74 \pm 1.03
	MPTP/vehicle	7.82 \pm 0.68
	MPTP/2-(phosphonomethyl)-	16.28 \pm 0.98
15	pentanedioic acid	

Mice treated with MPTP and vehicle exhibited a substantial loss of functional dopaminergic terminals as compared to non-lesioned mice (approximately 68% loss). Lesioned mice receiving 2-(phosphonomethyl)pentanedioic acid (10 mg/kg) showed a significant recovery of TH-stained dopaminergic neurons ($p < 0.001$). These results indicate that 2-(phosphonomethyl)pentanedioic acid protects against MPTP-toxicity in mice.

WO 98/13044

PCT/US97/14417

122

**Protocol for In Vivo Assay of NAALADase Inhibitors on
Parkinson's Disease**

MPTP lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's Disease, as described by Steiner, *Proc. Natl. Acad. Sci.*, Vol. 94, pp. 2019-2024 (March 1997). In brief, four week old male CD1 white mice were dosed IP with 30 mg/kg of MPTP for 5 days. 2-(Phosphonomethyl)pentanedioic acid (10 mg/kg) or a vehicle was administered SC along with the MPTP for 5 days, as well as for an additional 5 days following cessation of MPTP treatment. At 18 days following MPTP treatment, the mice were sacrificed and their brains were removed and sectioned. Immunostaining was performed on sagittal and coronal brain sections using anti-tyrosine hydroxylase (TH) antibodies to quantitate survival and recovery of dopaminergic neurons.

**In Vivo Assay of NAALADase Inhibitors on Dynorphin-
Induced Spinal Cord Injury**

To examine the neuroprotective effect of NAALADase inhibitors on excitotoxic spinal cord injury in vivo, rats which had sustained dynorphin-induced spinal cord injury were treated with a vehicle or 2-(phosphonomethyl)pentanedioic acid.

The results are graphically presented in FIG. 12. When co-administered with dynorphin A, 2-

WO 98/13044

PCT/US97/14417

123

(phosphonomethyl)pentanedioic acid (4 μ moles) caused significant improvement in motor scores by 24-hour post-injection, as compared to vehicle treated rats ($p < 0.05$, Kruskal-Wallis comparison). The rats were characterized as ambulatory or not on the basis of their assigned neurological scores (0 to 4). At 24 hours post-injection, 73% of the 15 rats co-treated with 2-(phosphonomethyl)pentanedioic acid were ambulatory, in contrast to 14% of the 14 vehicle co-treated rats ($p < 0.05$). These results indicate that 2-(phosphonomethyl)-pentanedioic acid provides effective protection against dynorphin-induced spinal cord injury.

Protocol for In Vivo Assay of NAALADase Inhibitors on
Dynorphin-Induced Spinal Cord Injury

Spinal Subarachnoid Injections

Dynorphin-induced spinal cord injury was performed according to Long et al., *JPET*, Vol. 269, No. 1, pp. 358-366 (1993). In brief, spinal subarachnoid injections were delivered using 30-gauge needles inserted between the L4-L5 vertebrae of male Sprague-Dawley rats (300-350 g). The rats were anesthetized with halothane and dorsal midline incisions were made immediately rostral to the pelvic girdle. By using the vertebral processes as guides, the needle was advanced to pass into the subarachnoid space surrounding the cauda equina. Correct

WO 98/13044

PCT/US97/14417

124

needle placement was verified by CSF flow from the needle after its insertion. Injections were delivered using a Hamilton microsyringe in a total volume of 20 μ l which contained dynorphin (20 nmol), the cannula flush and 2-
5 (phosphonomethyl)pentanedioic acid or vehicle. After injections, the incisions were treated with the topical antibacterial furazolidone and closed with wound clips. Rapid recovery from the halothane anesthesia enabled neurological evaluations to be made within 5 minutes of
10 injections.

Neurological Evaluations

Neurological function was evaluated using a 5-point ordinal scale, with scores being assigned as follows: 4 = normal motor function; 3 = mild paraparesis, with the
15 ability to support weight and walk with impairment; 2 = paraparesis, with the ability to make walking movements without fully supporting weight; 1 = severe paraparesis, in which rats could make limited hind limb movement, but not walking movement; and 0 = flaccid paralysis, with
20 complete absence of any hind limb movement. Neurological evaluations were made 24 hours after dynorphin A injection.

Statistics

Differences in the neurological scores among
25 treatment groups were determined by means of the Mann-Whitney U test or the Kruskal-Wallis test.

WO 98/13044

PCT/US97/14417

125

In Vitro Assay of NAALADase Inhibitors on Amyotrophic
Lateral Sclerosis (ALS)

To examine the neuroprotective effect of NAALADase inhibitors on Amyotrophic Lateral Sclerosis (ALS), spinal
5 cord organotypic cultures were treated with threohydroxyaspartate (THA), 2-(phosphonomethyl)-pentanedioic acid, or THA combined with 2-(phosphonomethyl)pentanedioic acid, and assayed for choline acetyltransferase (ChAT) activity.

10 The ChAT activity for each treatment of the spinal cord organotypic cultures is provided below in TABLE XI and graphically presented in FIG. 13.

WO 98/13044

PCT/US97/14417

126

TABLE XI

NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
SPINAL CORD CULTURE MODEL OF ALS

5	Treatment	ChAT Activity (% of Control)
	control	100 ± 22.1
10	2-(phosphonomethyl)- pentanedioic acid alone	108 ± 18.4
	THA alone	36 ± 12.1
15	2-(phosphonomethyl)- pentanedioic acid and THA	121 ± 18.8

20

As shown in FIG. 13, treatment of the spinal cord organotypic cultures with 100 μ M THA resulted in a reduction of ChAT activity to approximately 36% of control cultures. Co-incubation of the cultures with THA and 2-(phosphonomethyl)pentanedioic acid (100 nM - 10 μ M) significantly protected the cultures from THA toxicity.

25

WO 98/13044

PCT/US97/14417

127

The dose-response of this effect is provided below
in TABLE XII and graphically presented in FIG. 14.

TABLE XII

5 NEUROPROTECTIVE EFFECT OF NAALADASE INHIBITORS IN
 SPINAL CORD CULTURE MODEL OF ALS

10	control	ChAT Activity (% of Control)
	THA	0
15	THA and 1 nM 2-(phosphonomethyl)- pentanedioic acid	-23.9 ± 18.6
	THA and 10 nM 2-(phosphonomethyl)- pentanedioic acid	23.1 ± 12.5
20	THA and 100 nM 2-(phosphonomethyl)- pentanedioic acid	87.5 ± 21.7
	THA and 1 μM 2-(phosphonomethyl)- pentanedioic acid	187.7 ± 32.8
25		

WO 98/13044

PCT/US97/14417

128

THA and 10 μ M 2-(phosphonomethyl)-pentanedioic acid 128.7 \pm 17.2

5 Spinal cord cultures were incubated with various doses of 2-(phosphonomethyl)pentanedioic acid (1 nM to 10 μ M) in the presence of THA (100 μ M) for 14 days. As shown in FIG. 14, 2-(phosphonomethyl)pentanedioic acid exhibited dose-dependent protection against THA-induced
10 toxicity with maximal effects at 1 μ M.

**Protocol for In Vivo Assay of NAALADase Inhibitors on
Amyotrophic Lateral Sclerosis (ALS)**

Spinal Cord Organotypic Cultures

15 Organotypic cultures were prepared from lumbar spinal cord of 8 day old rats, as described by Rothstein et al., *J. Neurochem.*, Vol. 65, No. 2 (1995), and Rothstein et al., *Proc. Natl. Acad. Sci. USA*, Vol. 90, pp. 6591-6595 (July 1993). In brief, lumbar spinal cords
20 were removed and sliced into 300 μ M-thick-dorsal-ventral sections, and five slices were placed on Millipore CM semipermeable 30-mm-diameter membrane inserts. The inserts were placed on 1 ml of culture medium in 35-mm-diameter culture wells. Culture medium consisted of 50%
25 minimal essential medium and phosphate-free HEPES (25 mM), 25% heat-inactivated horse serum, and 25% Hanks'

WO 98/13044

PCT/US97/14417

129

balanced salt solution (GIBCO) supplemented with D-glucose (25.6 mg/ml) and glutamine (2 mM), at a final pH of 7.2. Antibiotic and antifungal agents were not used. Cultures were incubated at 37° C in 5% CO₂ containing humidified environment (Forma Scientific). Culture medium, along with any added pharmacological agents, was changed twice weekly.

Chronic Toxicity Model with THA

For all experiments, cultures were used 8 days after preparation at which time threohydroxyaspartate (THA; 100 μ M), 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μ M), or THA (100 μ M) \pm 2-(phosphonomethyl)pentanedioic acid (100 pM - 10 μ M) were added to the culture medium. Drugs were incubated for an additional 13 to 20 days with the 100 μ M THA. At the end of this period, cultures were collected assayed for ChAT activity as described below.

ChAT Assays

To determine choline acetyltransferase (ChAT) activity, the spinal cord tissues in each dish (five slices) were pooled and frozen (-75° C) until assay. ChAT activity was measured radiometrically by described methods using [³H]acetyl-CoA (Amersham; Fonnum, 1975). Protein content of tissue homogenate was determined by a Coomassie Protein Assay kit (Pierce, Rockford, IL).

25

WO 98/13044

PCT/US97/14417

130

In Vivo Assay of NAALADase Inhibitors on EthanolConsumption in Alcohol-Preferring Rats

To test the effect of NAALADase inhibitors on ethanol consumption, alcohol-preferring rats were treated with saline or a 50, 100 or 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid prior to ethanol access. The ethanol intake of the rats following treatment is graphically presented in FIG. 15.

As shown in FIG. 15, the 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid exhibited no effect, whereas both the 50 and 100 mg/kg doses significantly reduced ethanol consumption by approximately 25% ($p < 0.05$) during the 1 hour access period. Body weights and 24 hour water intakes were not altered at any of the 3 doses. If 2-(phosphonomethyl)pentanedioic acid is acting centrally, these data suggest that NAALADase may be involved in neuronal systems regulating alcohol-drinking behavior.

20 Saline Baseline: 8.9 ± 0.7

200 mg/kg 2-(phosphonomethyl)pentanedioic acid:

8 ± 0.5

Saline Baseline: 7.8 ± 0.8

25 100 mg/kg 2-(phosphonomethyl)pentanedioic acid:

5.8 ± 0.7

WO 98/13044

PCT/US97/14417

131

Saline Baseline: 8.1 ± 0.6

50 mg/kg 2-(phosphonomethyl)pentanedioic acid:

 6.2 ± 0.9

5 Protocol for In Vivo Assay of NAALADase Inhibitors on
 Ethanol Consumption in Alcohol-Preferring Rats

 The effect of systemic administration of 2-(phosphonomethyl)pentanedioic acid was examined on ethanol intake in the alcohol-preferring (P) line of
10 rats, as described by Panocka et al., *Pharm. Biochem. and Behavior*, Vol. 52, No. 2, pp. 255-259 (1995) and Murphy et al., *Alcohol*, Vol. 2, pp. 349-352 (1985). In brief, 2-(phosphonomethyl)pentanedioic acid (50, 100 and 200 mg/kg IP) was tested in female P rats (n = 8) given daily
15 1 hour scheduled access to a 10% (v/v) ethanol solution. A within-subject design was used where 2-(phosphonomethyl)pentanedioic acid treatments were tested once per week. Baseline ethanol drinking consisted of the mean of the 3 days prior to testing in which saline
20 injections were given. 2-(Phosphonomethyl)pentanedioic acid or saline, administered IP in 1 ml/kg volumes, were injected 10-15 minutes prior to ethanol access. 24 hour water and daily body weights were recorded to assess non-specific drug effects. Results were analyzed using
25 paired t-tests with baseline and test day values serving as the independent variables. Ethanol intake was

WO 98/13044

PCT/US97/14417

132

recorded as amount of solution consumed (mls).

In Vivo Assay of NAALADase Inhibitors on Nicotine Self-Administration in Male Long-Evans Rats

5 To test the effect of NAALADase inhibitors on nicotine self-administration, male Long-Evans rats trained to self-administer nicotine were treated with a 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid prior to nicotine access. The cumulative nicotine intake
10 of the rats following treatment is graphically presented in FIG. 16.

 The results show that the 200 mg/kg dose of 2-(phosphonomethyl)pentanedioic acid reduced nicotine self-administration from 23 to 5 infusions during the 1 hour
15 access period. As graphically presented in FIG. 17, the cumulative food intake of the rats also decreased during the same period of time. While these data suggest that factors other than 2-(phosphonomethyl)pentanedioic acid may be responsible for the reduction in nicotine self-
20 administration, they do not disprove NAALADase's involvement in the neuronal systems regulating nicotine use. The effect on the rats' food intake could be attributed to toxicity caused by an excessive drug dose.

25

WO 98/13044

PCT/US97/14417

133

Protocol for In Vivo Assay of NAALADase Inhibitors on
Nicotine Self-Administration in Male Long-Evans Rats

Male Long-Evans rats were trained to self-administer nicotine on a fixed ratio schedule of reinforcement, as described by Corrigan et al., *Psychopharmacology*, Vol. 104, No. 2, pp. 171-176 (1991) and Corrigan et al., *Psychopharmacology*, Vol. 107, Nos. 2-3, pp. 285-289 (1992). In brief, male Long-Evans rats were food deprived for a short period of time (24-48 hours) and trained to press a lever in an operant responding chamber on an FR-1 schedule of food reinforcement. Once trained, each rat was surgically prepared with a chronic intravenous catheter implanted into the jugular vein. The rats were allowed 1 week to recover from surgery.

After 1 week, nicotine self-administration studies were initiated on an FR-1 with a 60 second signaled time-out following each infusion. During time-out, responding on the lever had no scheduled consequence. Nicotine self-administration sessions were 60 minutes in duration. Each nicotine infusion contained 30 μ g of nicotine/kg rat and were delivered in a volume of 54 μ l over an infusion duration of 0.3 seconds. 15 minutes before the self-administration sessions, the rats were pre-treated intraperitoneally with 2-(phosphonomethyl)-pentanedioic acid at doses of 10, 20 and 30 mg/kg. Food intake was monitored during the nicotine self-administration

WO 98/13044

PCT/US97/14417

134

sessions to assess non-specific drug effects.

EXAMPLES

The following examples are illustrative of the present invention and are not intended to be limitations thereon. Unless otherwise indicated, all percentages are based upon 100% by weight of the final composition.

EXAMPLE 1

Preparation of 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV: $R = CH_3$, $R_1 = CH_2Ph$

Methyl-O-benzylphosphinic acid

Dichloromethylphosphite (10.0 g, 77 mmol) in 80 mL of dry diethyl ether was cooled to $-20^{\circ}C$ under an atmosphere of nitrogen. A solution of benzyl alcohol (23 g, 213 mmol) and triethylamine (10.2 g, 100 mmol) in 40 mL of diethyl ether was added dropwise over 1 hour while maintaining an internal temperature range of $0^{\circ}C$ to $10^{\circ}C$. Once addition was complete the mixture was warmed to room temperature and stirred overnight. The mixture was filtered and the solid cake washed with 200 mL of diethyl ether. The organics were combined and evaporated under reduced pressure to give 25 g of a clear and colorless liquid. The liquid was purified by flash chromatography

WO 98/13044

PCT/US97/14417

135

and eluted with a 1:1 hexane/ethyl acetate to ethyl acetate gradient. The desired fractions were collected and evaporated to give methyl-O-benzylphosphinic acid (1, R = CH₃, R₁ = CH₂Ph, 6.5 g, 50%) as a clear and colorless oil. R_f 0.1 (1:1, Hexane/EtOAc).

¹H NMR (d₆-DMSO): 7.4 ppm (m, 5H), 7.1 ppm (d, 1H), 5.0 ppm (dd, 2H), 1.5 ppm (d, 3H)

2,4-Di(benzyloxycarbonyl)butyl(methyl)-O-benzylphosphinic
10 acid

Methyl-O-benzylphosphinic acid (3.53 g, 20.7 mmol) in 200 mL of dichloromethane was cooled to -5° C under an atmosphere of nitrogen. Triethylamine (3.2 g, 32 mmol) was added via syringe followed by trimethylsilyl chloride
15 (2.9 g, 27 mmol). The reaction mixture was stirred and warmed to room temperature over 1 hour. Dibenzyl 2-methylenepentanedioate (2, 6.0 g, 18.5 mmol) in 10 mL of dichloromethane was added. The mixture was then stirred at room temperature overnight. The reaction mixture was
20 cooled to 0° C and trimethylaluminum (9 mL, 18 mmol, 2.0 M in dichloromethane) was added. The flask was warmed and stirred for 72 hours. The clear light yellow solution was cooled to 5° C and quenched by the slow addition of 5% hydrochloric acid. The quenched reaction
25 mixture was warmed to room temperature and the organic layer removed. The organic layer was washed with 5%

WO 98/13044

PCT/US97/14417

136

hydrochloric acid and with water. The organics were dried (MgSO_4) and evaporated under reduced pressure to give 8 g of a clear light yellow oil. The oil was purified on silica gel and eluted with a gradient of 1:1
5 hexanes/ethyl acetate to 100% ethyl acetate. The desired fractions were collected and evaporated to give 2,4-di(benzyloxycarbonyl)butyl(methyl)-O-benzylphosphinic acid (3, R = CH_3 , $\text{R}_1 = \text{CH}_2\text{Ph}$, 0.8 g, 8%) as a clear and colorless oil. R_f 0.5 (ethyl acetate).
10 ^1H NMR (CDCl_3): 7.4 ppm (m, 15H), 5.1 ppm (m, 6H), 3.0 ppm (m, 1H), 2.4 ppm (m, 3H), 2.1 ppm (m, 3H), 1.5 ppm (dd, 3H).

Elemental Analysis

Calculated $\text{C}_{28}\text{H}_{31}\text{O}_6\text{P}$, 0.5 H_2O : C, 68.01; H, 6.32.

15 Found: C, 66.85; H, 6.35.

2-[(Methylhydroxyphosphinyl)methyl]pentanedioic acid

2,4-di(benzyloxycarbonyl)butyl(methyl)-O-benzylphosphinic acid (0.8 g, 1.6 mmol) in 20 mL of water
20 containing 100 mg of 10% Pd/C was hydrogenated at 40 psi for 4 hours. The mixture was filtered over a pad of Celite and evaporated at high vacuum to give 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid (4, R = CH_3 , 0.28 g), 78% as a clear and colorless viscous oil.
25 ^1H NMR (D_2O): 2.5 ppm (m, 1H), 2.2 ppm (t, 2H), 2.0 ppm (m, 1H), 1.7 ppm (m, 3H), 1.3 ppm (d, 3H).

WO 98/13044

PCT/US97/14417

137

Elemental Analysis

Calculated $C_7H_{13}O_6P$, 0.2 H_2O : C, 36.92; H, 5.93.

Found: C, 37.06; H, 6.31.

5

EXAMPLE 2

Preparation of 2-[(butylhydroxyphosphinyl)methyl]
pentanedioic acid

Scheme IV: R = n-butyl, R_1 = H

10 Butylphosphinic Acid

Diethyl chlorophosphite (25 g, 0.16 mol) in 60 mL of dry ether was cooled to 0° C under an atmosphere of nitrogen. Butylmagnesium chloride (80 mL, 0.16 mol, 2.0 M solution in ether) was added dropwise over a period of 2 hours while maintaining the internal temperature at 0° C. Once addition was complete the thick white slurry was heated to 30° C for 1 hour. The suspension was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure. The clear light yellow liquid was then brought up in 15 mL of water and stirred at room temperature. Concentrated hydrochloric acid (0.5 mL) was then added and an exothermic reaction was observed. The mixture was stirred an additional 15 minutes and extracted with two 75 mL portions of ethyl acetate. The organics were combined, dried ($MgSO_4$) and evaporated to give a clear and colorless liquid. The liquid was

25

WO 98/13044

PCT/US97/14417

138

treated with NaOH (40 mL, 2.0 M) and stirred for 1 hour. The mixture was then washed with diethyl ether and acidified to pH 1.0. The desired material was extracted from the acidified extract with two 100 mL portions of
5 ethyl acetate. The organics were combined, dried (MgSO₄) and evaporated under reduced pressure to give butylphosphinic acid (1, R = n-butyl, R₁ = H, 10 g, 51%) as a clear and colorless liquid.

¹H NMR (d₆-DMSO): 6.9 ppm (d, 1H), 1.6 ppm (m, 2H), 1.4
10 ppm (m, 4H), 0.9 ppm (t, 3H).

Butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid

Butylphosphinic acid (2.0 g, 16 mmol) in 80 mL of dry dichloromethane was cooled to 0° C under an
15 atmosphere of nitrogen. Triethylamine (6.7 g, 66 mmol) was added followed by trimethylsilyl chloride (58 mL, 58 mmol, 1.0 M in dichloromethane). The mixture was stirred at 0° C for 10 minutes and dibenzyl 2-methylenepentanedioate (2) (6.4 g, 20 mmol) in 20 mL of
20 dichloromethane was added. The cold bath was removed and the reaction warmed to room temperature and stirred overnight. The mixture was then cooled to 0° C and quenched by the slow addition of 5% hydrochloric acid (50 mL). The dichloromethane layer was then removed and
25 washed with 5% hydrochloric acid and with brine. The organic layer was dried (MgSO₄) and evaporated to give a

WO 98/13044

PCT/US97/14417

139

clear light golden liquid. The liquid was purified by flash chromatography and eluted with 3:1 hexane/ethyl acetate containing 5% acetic acid. The desired fractions were combined and evaporated to give butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (3, R = n-butyl, R₁ = H) (2.9 g, 40%) as a clear and colorless oil. R_f 0.12 (3:1 Hexane/EtOAc, 5% AcOH).

¹H NMR (d₆-DMSO): 7.3 ppm (m, 10H), 5.0 ppm (s, 4H), 2.7 ppm (m, 1H), 2.3 ppm (t, 2H), 1.8 ppm (m, 2H), 1.3 ppm (m, 4H), 0.8 ppm (t, 3H).

2-[(Butylhydroxyphosphinyl)methyl]pentanedioic acid

Butyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (2.9 g, 6.5 mmol) in 30 mL of water containing 0.32 g 10% Pd/C was hydrogenated at 40 psi for 4.5 hours. The mixture was filtered through a pad of Celite and evaporated under high vacuum to give 2-[(butylhydroxyphosphinyl)methyl]-pentanedioic acid (4, R = n-butyl) (0.75 g, 43%) as a clear and colorless viscous oil.

¹H NMR (D₂O): 2.4 ppm (m, 1H), 2.1 ppm (t, 2H), 1.9 ppm (m, 1H), 1.6 ppm (m, 3H), 1.4 ppm (m, 2H), 1.1 ppm (m, 4H), 0.6 ppm (t, 3H).

Elemental Analysis

Calculated C₁₀H₁₉O₆P, 0.5 H₂O: C, 43.64; H, 7.32.

Found: C, 43.25; H, 7.12.

WO 98/13044

PCT/US97/14417

140

EXAMPLE 3

Preparation of 2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acidScheme IV: R = CH₂Ph, R₁ = H

5

Benzylphosphinic acid

Diethylchlorophosphite (25 g, 0.16 mol) in 100 mL of dry diethyl ether was cooled to 0° C under an atmosphere of nitrogen. Benzylmagnesium chloride (80 mL, 0.16 mol, 2.0 M solution in Et₂O) was added dropwise over two hours while maintaining a temperature below 10° C. A thick white slurry formed and stirring was continued at room temperature for 1 hour. The mixture was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure to give a clear and colorless liquid. The liquid was stirred as 15 mL of water was added followed by 0.5 ml concentrated hydrochloric acid. An exothermic reaction was observed and stirring was continued for an additional 30 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO₄) and evaporated. The clear light golden liquid was added to sodium hydroxide (50 mL, 2.0 M NaOH), stirred for 1 hour and washed with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate. The organics were

WO 98/13044

PCT/US97/14417

141

combined, dried (MgSO_4) and evaporated to give benzylphosphinic acid (1, $\text{R} = \text{CH}_2\text{Ph}$, $\text{R}_1 = \text{H}$) (8 g, 32%) as a clear light golden oil.

^1H NMR (d_6 -DMSO): 7.3 ppm (m, 5H), 6.9 ppm (d, 1H), 3.1
5 ppm (d, 2H).

Benzyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid

Benzylphosphinic acid (2.3 g, 15 mmol) in 150 mL of dry dichloromethane was cooled to 0°C under a nitrogen
10 atmosphere. Triethylamine (6.5 g, 65 mmol) was added followed by trimethylsilyl chloride (5.8 g, 54 mmol) while the reaction temperature was maintained at 0°C . After 30 minutes dibenzyl 2-methylene-pentanedioate
(2) (4.4 g, 13.6 mmol) in 20 mL of dichloromethane was
15 added over 5 minutes. The reaction mixture was allowed to warm to room temperature and stirred overnight. The clear solution was cooled to 0°C and quenched with 5% hydrochloric acid followed by removal of the organic layer. The organic layer was washed with 5% hydrochloric
20 acid and with brine, dried (MgSO_4) and evaporated to give a clear yellow liquid. Purification by flash chromatography and elution with 1:1 hexane/ethyl acetate containing 10% acetic acid yielded 2.0 g (28%) of
25 benzyl[2,4-di(benzyloxycarbonyl)butyl]-phosphinic acid (3, $\text{R} = \text{CH}_2\text{Ph}$, $\text{R}_1 = \text{H}$) as a clear light yellow oil. R_f 0.37 (1:1 Hexane/EtOAc, 10% AcOH).

WO 98/13044

PCT/US97/14417

142

¹H NMR (d6-DMSO): 7.2 ppm (m, 15H), 5.0 ppm (s, 4H), 3.0 (d, 2H), 2.8 ppm (m, 1H), 2.3 ppm (t, 2H), 1.9 ppm (m, 2H), 1.7 ppm (t, 1H).

5 2-[(Benzylhydroxyphosphinyl)methyl]pentanedioic acid

Benzyl[2,4-di(benzyloxycarbonyl)butyl]phosphinic acid (0.5 g, 1.0 mmol) in 20 mL of water containing 120 mg of 10% Pd/C was hydrogenated at 40 psi for 6 hours. Filtration through a Celite pad followed by evaporation
10 on high vacuum gave 0.17 g (57%) of 2-[(benzylhydroxyphosphinyl)methyl]-pentanedioic acid (4, R = CH₂Ph) as a white solid.

¹H NMR (D₂O): 7.1 ppm (m, 5H), 2.9 ppm (d, 2H), 2.4 ppm (m, 1H), 2.1 ppm (t, 2H), 1.8 ppm (m, 1H), 1.6 ppm (m,
15 3H).

Elemental Analysis

Calculated C₁₁H₁₇O₆P: C, 52.00; H, 5.71.

Found: C, 51.48; H, 5.70.

20

EXAMPLE 4

Preparation of 2-

[phenylethylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV: R = CH₂CH₂Ph, R₁ = H

25 Phenethylphosphinic acid

Diethylchlorophosphite (15.6 g, 0.1 mol) in 100 mL

WO 98/13044

PCT/US97/14417

143

of dry diethyl ether was cooled to 5° C under an atmosphere of nitrogen. Phenethylmagnesium chloride (100 mL, 0.1 mol, 1.0 M in THF) was added dropwise over 2 hours while maintaining a temperature between 0-10° C. A thick white slurry formed and stirred at room temperature overnight. The mixture was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure to give a clear and colorless liquid. The liquid was stirred as 15 mL of water was added followed by 0.5 mL of concentrated hydrochloric acid. An exothermic reaction was observed and stirring continued for 15 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO₄) and evaporated. The clear liquid was brought up in sodium hydroxide (40 mL, 2.0 M NaOH), stirred for 1 hour and washed once with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted with ethyl acetate. The organics were combined, dried (MgSO₄) and evaporated to give phenethylphosphinic acid (1, R = CH₂CH₂Ph, R₁ = H) (9.8 g, 58%) as a clear light yellow oil.

¹H NMR (d₆-DMSO): 7.2 ppm (m, 5H), 6.9 ppm (d, 1H), 2.8 ppm (m, 2H), 1.9 ppm (m, 2H).

2,4-Di(benzyloxycarbonyl)butyl(phenethyl)phosphinic acid
Phenethylphosphinic acid (1.0 g, 5.9 mmol) in 50 mL

WO 98/13044

PCT/US97/14417

144

of dry dichloromethane was cooled to -5°C under a nitrogen atmosphere. Triethylamine (2.3 g, 23 mmol) was added followed by trimethylsilyl chloride (2.2 g, 21 mmol) while the reaction temperature was maintained at 0°C . After 10 minutes dibenzyl 2-methylenepentanedioate (2) (1.7 g, 5.2 mmol) in 10 mL of dichloromethane was added over 10 minutes. The reaction mixture was left to warm to room temperature and stirred overnight. The clear solution was cooled to 0°C and quenched with 5% hydrochloric acid followed by removal of the organic layer. The organic layer was washed with brine, dried (MgSO_4) and evaporated to give a clear light golden liquid. Purification by flash chromatography and elution with 1:1 Hexane/EtOAc containing 5% AcOH yielded 1.2 g (41%) of 2,4-di(benzyloxycarbonyl)-butyl(phenethyl)phosphinic acid (3, $\text{R} = \text{CH}_2\text{CH}_2\text{Ph}$, $\text{R}_1 = \text{H}$) as a clear and colorless oil.

^1H NMR (d_6 -DMSO): 7.2 ppm (m, 15H), 5.0 ppm (s, 4H), 3.3 ppm (m, 1H), 2.8 ppm (m, 4H), 2.3 ppm (m, 2H), 1.8 ppm (m, 4H).

2 - [(Phenethylhydroxyphosphinyl)methyl]pentanedioic acid

2,4-Di(benzyloxycarbonyl)butyl(phenethyl)-phosphinic acid (1.1 g, 2.2 mmol) in 20 mL of water containing 120 mg of 10% Pd/C was hydrogenated at 40 psi overnight. Filtration through a Celite pad followed by evaporation

WO 98/13044

PCT/US97/14417

145

on high vacuum gave 0.8 g (114%) of 2-
[(phenethylhydroxyphosphinyl)methyl]pentanedioic acid (4,
R = CH₂CH₂Ph) as a white solid.

¹H NMR (D₂O): 7.2 ppm (m, 5H), 2.7 ppm (m, 2H), 2.5 ppm
5 (m, 1H), 2.3 ppm (t, 2H), 1.9 ppm (m, 6H), 1.5 ppm (t, 1H)

Elemental Analysis

Calculated C₁₄H₁₉O₆P, 0.75H₂O, 0.5 AcOH: C, 50.35; H,
6.34.

Found: C, 50.26; H, 5.78.

10

EXAMPLE 5

Preparation of 2-[(3-

phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid

Scheme IV: R = CH₂CH₂CH₂Ph, R₁ = H

15

3-Phenylpropylphosphinic acid

To magnesium turnings (2.44 g, 0.10 mol) in 20 mL of dry
diethyl ether under an atmosphere of nitrogen was added
several iodine crystals. Phenylpropyl bromide (20.0 g,
20 0.10 mol) in 80 mL of diethyl ether was placed in a
dropping funnel. Approximately 10 mL of the bromide
solution was added to the magnesium turnings and stirring
was initiated. After several minutes the iodine was
consumed and additional phenylpropyl bromide was added
25 while maintaining a temperature of 35° C. Once addition
was complete (1.5 hours) the mixture was sealed and

WO 98/13044

PCT/US97/14417

146

stored at 5° C.

Diethylchlorophosphite (15.7 g, 0.1 mol) in 50 mL of dry diethyl ether was cooled to 5° C under an atmosphere of nitrogen. Phenethylmagnesium bromide (100 mL, 0.1 mol, 1.0 M solution of in Et₂O) was added dropwise over 2 hours while maintaining a temperature between 0 - 10° C. A thick white slurry formed and was stirred for an additional 30 minutes. The mixture was filtered under a nitrogen atmosphere and the filtrate evaporated under reduced pressure to give a clear and colorless liquid. To the liquid was added 20 mL of water followed by 0.5 ml of concentrated hydrochloric acid. An exothermic reaction was observed and stirring continued for 20 minutes followed by extraction with ethyl acetate. The organics were combined, washed with brine, dried (MgSO₄) and evaporated. To the clear liquid was added sodium hydroxide (40 mL, 2.0 M NaOH), the resulting solution stirred for 1 hour and then washed with diethyl ether. The aqueous layer was acidified to pH 1.0 with concentrated hydrochloric acid and extracted twice with ethyl acetate. The organics were combined, dried (MgSO₄) and evaporated to give 3-phenylpropylphosphinic acid (1, R = CH₂CH₂CH₂Ph, R₁ = H) (9.8 g, 53%) as a clear and colorless oil.

¹H NMR (d₆-DMSO): 7.2 ppm (m, 5H), 6.9 ppm (d, 1H), 2.6 ppm (t, 2H), 1.7 ppm (m, 2H), 1.6 ppm (m, 2H).

WO 98/13044

PCT/US97/14417

147

2,4-Di(benzyloxycarbonyl)butyl(3-phenylpropyl)-phosphinic acid

3-phenylpropylphosphinic acid (1.0 g, 5.4 mmol) in 50 mL of dry dichloromethane was cooled to -5° C under a nitrogen atmosphere. Triethylamine (2.2 g, 22 mmol) was added followed by trimethylsilyl chloride (2.1 g, 19 mmol) while the reaction temperature was maintained at 0° C. After 10 minutes dibenzyl 2-methylenepentanedioate (2) (1.6 g, 4.9 mmol) in 10 mL of dichloromethane was added over 10 minutes. The reaction mixture was warmed to room temperature and stirred overnight. The clear solution was cooled to 0° C and quenched with 5% hydrochloric acid followed by removal of the organic layer. The organic layer was washed with brine, dried (MgSO₄) and evaporated to give a clear yellow liquid. Purification by flash chromatography and elution with 4:1 hexane/ethyl acetate containing 5% acetic acid resulted in 1.5 g (56%) of 2,4-di(benzyloxycarbonyl)-butyl(3-phenylpropyl)phosphinic acid (3, R = CH₂CH₂CH₂Ph, R₁ = H) as a clear light yellow oil. R_f 0.58 (1:1 Hexane/EtOAc, 5% AcOH).

¹H NMR (d₆-DMSO): 7.2 ppm (m, 15H), 5.0 ppm (s, 4H), 2.7 ppm (m, 1H), 2.5 ppm (m, 5H), 2.2 ppm (m, 2H), 1.8 ppm (m, 3H), 1.6 ppm (m, 2H).

Elemental Analysis

Calculated C₂₉H₃₃O₆P, 1.3 H₂O: C, 65.48; H 6.75.

WO 98/13044

PCT/US97/14417

148

Found: C, 65.24; H, 6.39.

2-[(3-Phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid

5 2,4-Di(benzyloxycarbonyl)butyl(3-phenylpropyl)phosphinic acid (15) (1.4 g, 2.8 mmol) in 20 mL of water containing 150 mg of 10% Pd/C was hydrogenated at 40 psi overnight. Filtration through a Celite pad followed by evaporation on high vacuum gave
10 0.8 g (89%) of 2-[(3-phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid (4, R = CH₂CH₂CH₂Ph) as a light yellow viscous oil.

¹H NMR (D₂O): 7.4 ppm (m, 5H), 2.7 ppm (m, 3H), 2.4 ppm (t, 3H), 1.8 ppm (m, 7H).

15 **Elemental Analysis**

Calculated C₁₅H₂₁O₆P, 0.75 H₂O, 0.75 AcOH: C, 51.23; H, 6.64.

Found: C, 50.85; H, 6.02.

20

EXAMPLE 6

Preparation of 2-[[[4-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic acid

Scheme V: Compound 5, R = 4-methylbenzyl

Hexamethyldisilazane (21.1 mL, 100 mmol) was added to
25 vigorously stirred ammonium phosphinate (8.30 g, 100 mmol), and the resulting suspension was stirred at 105°

WO 98/13044

PCT/US97/14417

149

C for 2 hours. A solution of 4-methylbenzyl bromide (5.0 g, 27.0 mmol) was then added dropwise to the suspension at 0° C. The mixture was stirred at room temperature for 19 hours. The reaction mixture was then diluted with dichloromethane (50 mL) and washed with 1 N HCl (50 mL). The organic layer was separated, dried over Na₂SO₄, and concentrated to give 4.72 g of a white solid. This was dissolved in dichloromethane (50 mL) and benzyl alcohol (3.24 g, 30 mmol) was added to the solution. 1,3-Dicyclohexylcarbodiimide (DCC) (6.19 g, 30 mmol) was then added to the solution at 0° C, and the suspension was stirred at room temperature for 14 hours. The solvent was removed under reduced pressure and the residue was suspended in EtOAc. The resulting suspension was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (hexanes: EtOAc, 4:1 to 1:1) to give 2.40 g of 4-methylbenzyl-O-benzylphosphinic acid (2, R = 4-methylbenzyl) as a white solid (34% yield). R_f 0.42 (EtOAc).

¹H NMR (DMSO-d₆): δ 2.30 (s, 3H), 3.29 (d, 2H), 5.2 (m, 2H), 7.0 (d, 1H), 7.1-7.2 (m, 4H), 7.3-7.4 (m, 5H).

2,4-Di(benzyloxycarbonyl)-butyl(4-methylbenzyl)-o-benzylphosphinic acid

To a solution of 4-methylbenzyl-O-benzylphosphinic acid (2, R = 4-methylbenzyl) (2.16 g, 8.3 mmol) in THF (15

WO 98/13044

PCT/US97/14417

150

mL) was added sodium hydride (0.10 g, 60% dispersion in oil) followed by dibenzyl 2-methylenepentanedioate (3) (3.24 g) at 0° C, and the mixture was stirred at room temperature for 4 hours. The reaction mixture was then
5 diluted with EtOAc (50 mL) and poured into 1 N HCl (50 mL). The organic layer was separated, dried over Na₂SO₄, and concentrated. This material was purified by silica gel chromatography (hexanes: EtOAc, 4:1 to 1:1) to give
3.41 g of 2,4-di(benzyloxycarbonyl)-butyl(4-methylbenzyl)-o-benzylphosphinic acid (4, R = 4-methylbenzyl) as colorless oil (70% yield). R_f 0.61 (EtOAc).

¹H NMR (CDCl₃): δ 1.6-1.8 (m, 1H), 1.9-2.0 (m, 2H), 2.1-2.4 (m, 6H), 2.7-2.9 (m, 1H), 3.05 (dd, 2H), 4.8-5.1 (m,
15 6H), 7.0-7.1 (m, 4H), 7.2-7.4 (m, 15H).

2-[[[(4-Methylbenzyl)hydroxyphosphinyl)methyl]-pentanedioic acid

To a solution of 2,4-di(benzyloxycarbonyl)butyl(4-methylbenzyl)-o-benzylphosphinic acid (0.70 g, 1.2 mmol)
20 in ethanol (30 mL) was added Pd/C (5%, 0.10 g) and the suspension was shaken under hydrogen (50 psi) for 18 hours. The suspension was then filtered through a pad of Celite and concentrated under reduced pressure. The
25 resulting residue was dissolved in distilled water (5 mL), passed through a column of AG 50W-X8 resin (H'

WO 98/13044

PCT/US97/14417

151

form), and lyophilized to give 0.21 g of 2-[[[4-methylbenzyl)hydroxyphosphinyl)methyl]-pentanedioic acid (5, R = 4-methylbenzyl) as a white solid (55% yield). *R_f* 0.62 (*i*-PrOH: H₂O, 7:3).

5 ¹H NMR (D₂O): δ 1.7-1.9 (m, 3H), 2.0-2.2 (m, 1H), 2.33 (dt, 7.4 Hz, 2H), 2.55-2.70 (m, 1H), 3.12 (d, 2H), 7.0-7.1 (m, 2H), 7.2-7.3 (m, 2H). Elemental Analysis
Calculated C₁₃H₁₇O₆P, 0.30 H₂O: C, 52.60; H, 6.18. Found: C, 52.60; H, 6.28.

10

EXAMPLE 7

**Preparation of 2-[[[4-Fluorobenzyl)hydroxyphosphinyl]
methyl]pentanedioic acid**

Scheme V: R = 4-fluorobenzyl

15 Prepared as described in the above example where R = methylbenzyl. *R_f* 0.64 (*i*-PrOH:H₂O, 7:3).

¹H NMR (D₂O): δ 1.7-1.9 (m, 3H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 2H), 2.55-2.70 (m, 1H), 3.12 (d, 2H), 7.0-7.1 (m, 2H), 7.2-7.3 (m, 2H).

20 Elemental Analysis

Calculated C₁₃H₁₆FO₆P, 0.25 H₂O: C, 48.38; H, 5.15. Found: C, 48.38; H, 5.15.

25

WO 98/13044

PCT/US97/14417

152

EXAMPLE 8**Preparation of 2-[[(4-Methoxybenzyl)hydroxyphosphinyl]
methyl]pentanedioic acid**

Scheme V: R = 4-methoxybenzyl

5 Prepared as described in the above example where R = methylbenzyl. R_f 0.56 (*i*-PrOH: H₂O, 7:3).

¹H NMR (D₂O): δ 1.8-1.9 (m, 3H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 2H), 2.55-2.70 (m, 1H), 3.16 (d, 2H), 3.81 (s, 3H), 6.98 (d, 2H), 7.25 (d, 2H).

10 **Elemental Analysis**

Calculated C₁₄H₁₉O₇P, 0.30 H₂O: C, 50.09; H, 5.89.

Found: C, 49.98; H, 5.80.

EXAMPLE 9

15 **Preparation of 2-[[(2-Fluorobenzyl)hydroxyphosphinyl]
methyl]pentanedioic acid**

Scheme V: R = 2-fluorobenzyl)

Prepared as described in the above example where R = methylbenzyl. R_f 0.67 (*i*-PrOH: H₂O, 7:3).

20 ¹H NMR (D₂O): δ 1.8-1.9 (m, 3H), 2.0-2.2 (m, 1H), 2.3-2.4 (m, 2H), 2.55-2.70 (m, 1H), 3.28 (d, 2H), 7.1-7.5 (m, 4H).

Elemental Analysis

Calculated C₁₃H₁₆FO₆P, 0.10 H₂O: C, 48.79; H, 5.10. Found:

25 C, 48.84; H, 5.14.

WO 98/13044

PCT/US97/14417

153

EXAMPLE 10

Preparation of 2-[[(Pentafluorobenzyl)hydroxyphosphinyl]
methyl]pentanedioic acid

5 Scheme V: R = pentafluorobenzyl

Prepared as described in the above example where R =
methylbenzyl. R_f 0.69 (i-PrOH: H₂O, 7:3).

¹H NMR (D₂O): δ 1.8-2.0 (m, 3H), 2.1-2.3 (m, 1H), 2.3-
2.5 (m, 2H), 2.7-2.9 (m, 1H), 3.29 (d, 2H).

10 Elemental Analysis

Calculated C₁₁H₁₂F₅O₆P, 0.45 H₂O: C, 39.20; H, 3.26.

Found: C, 39.17; H, 3.28.

EXAMPLE 11

15 Preparation of 2-[(methylhydroxyphosphinyl)methyl]
pentanedioic acid

Scheme VI, Compound 9

2,4-Di(benzyloxycarbonyl)butylphosphinic acid (6)

20 Ammonium phosphinate (10 g, 0.12 mol) was placed in
a round bottom flask with stirring under an atmosphere of
nitrogen. Hexamethyldisilazane (HMDS, 25.5 mL, 0.12 mol)
was added and the mixture heated to 110° C. After two
hours the mixture was cooled to 0° C and dichloromethane
25 (120 ml) was added. After this was complete, dibenzyl-2-
methylene pentanedioate (41 g, 0.13 mol) was added

WO 98/13044

PCT/US97/14417

154

dropwise. The mixture was allowed to warm to room temperature and stirred for 16 hours. The mixture was then quenched with 5% HCl (75 ml) and the organic layer removed. The organics were dried (MgSO_4) and evaporated under reduced pressure to give 42 g (90%) of a clear and colorless oil.

^1H NMR (CDCl_3): 7.36 ppm (m, 10H), 7.1 ppm (d, 1H), 5.19 ppm (s, 2H), 5.15 ppm (s, 2H), 2.92 ppm (m, 1H), 2.21 ppm (m, 6H).

10

2,4-Di(benzyloxycarbonyl)butylbenzylphosphinic acid (7)

To a solution of 2,4-di-(benzyloxycarbonyl)butylphosphinic acid (6) (19.3 g, 49.4 mmol) in tetrahydrofuran was added benzyl alcohol (5.3 g, 49.3 mmol) and dimethylamino pyridine (0.5 g). Dicyclohexylcarbodiimide (DCC, 12 g, 58 mmol) was added and a white precipitate formed. After 30 minutes the white suspension was filtered and the filtrate evaporated under reduced pressure. The clear and colorless oil was purified by flash chromatography and eluted with 1:1 Hexane/EtOAc to give 2,4-di(benzyloxycarbonyl)butylbenzylphosphinic acid (7) (11.5 g, 47%) as a clear and colorless oil. R_f 0.16 (1:1 Hexane/EtOAc).

^1H NMR (CDCl_3): 7.3 ppm (m, 15H), 7.2 ppm (d, 1H), 5.0 ppm (m, 6H), 2.9 ppm (m, 1H), 2.2 ppm (m, 3H), 1.9 ppm (m, 3H).

25

WO 98/13044

PCT/US97/14417

155

2,4-Di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]benzylphosphinic acid (8)

2,4-Di(benzyloxycarbonyl)butylbenzylphosphinic acid (7) in 5 mL of dry THF was added dropwise to a stirring cooled (0° C) mixture of sodium hydride (0.09 g, 2.3 mmol) in 15 mL of THF. After 15 minutes benzaldehyde (0.23 g, 2.2 mmol) was added via syringe while maintaining a temperature of 0° C. After 30 minutes the mixture was quenched with water and extracted with two portions of dichloromethane. The organics were combined and evaporated to give a clear colorless oil. The oil was chromatographed on silica and eluted with a 1:1 Hexane/EtOAc solvent system. The desired fractions were collected and evaporated to give 0.4 g (33%) of 2,4-di(benzyloxycarbonyl)butyl-[hydroxy(phenyl)methyl]benzylphosphinic acid (6) as a clear and colorless oil. R_f 0.18 (1:1 Hexane/EtOAc). 1H NMR ($CDCl_3$): 7.3 ppm (m, 20H), 5.2 ppm (m, 1H), 4.9 ppm (m, 6H), 2.8 ppm (dm, 1H), 2.2 ppm (m, 3H), 1.9 ppm (m, 3H).

2-([Hydroxy(phenyl)methyl]hydroxyphosphinylmethyl)-pentanedioic acid (9)

2,4-Di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]benzylphosphinic acid (6) (0.37 g, 0.6 mmol) in 25 mL of water containing 0.10 g of 10% Pd/C was

WO 98/13044

PCT/US97/14417

156

hydrogenated at 40 psi for 6 hours. The mixture was filtered through a pad of Celite and lyophilized to give 2-([hydroxy(phenyl)methyl]hydroxyphosphinyl-methyl)pentanedioic acid (9) (0.14 g, 70%) as a white

5 solid.

¹H NMR (D₂O): 7.4 ppm (m, 5H), 5.0 ppm (d, 1H), 2.7 ppm (m, 1H), 2.4 ppm (m, 2H), 2.2 ppm (m, 1H), 1.9 ppm (m, 3H).

Elemental Analysis:

10 Calculated C₁₃H₁₇O₇P, 0.6 H₂O: C, 47.74; H, 5.61.

Found: C, 47.73; H, 5.68.

EXAMPLE 12

Preparation of dibenzyl 2-methylenepentanedioate using

15

Scheme III

Benzyl acrylate (500 g, 3.0 mol) was heated in an oil bath to 100° C. Heating was stopped and HMPT (10 g, 61 mmol) was added dropwise while maintaining an internal temperature below 140° C. Once addition was complete, the mixture was stirred and cooled to room temperature. A slurry of silica (5:1 Hexane/EtOAc) was added and the mixture was placed in a column containing a plug of dry silica. The column was washed with 1:1 Hexane/EtOAc and the fractions were combined and evaporated to give 450 g of clear light golden liquid. The liquid was distilled under high vacuum (200 μHg) at 185° C to give 212 g (42%)

20

25

WO 98/13044

PCT/US97/14417

157

of a clear and colorless liquid.

¹H NMR (CDCl₃): 7.3 ppm (m, 10H), 6.2 ppm (s, 1H), 5.6 ppm (s, 1H), 5.2 ppm (s, 2H), 5.1 ppm (s, 2H), 2.6 ppm (m, 4H).

5

EXAMPLE 13

Preparation of dibenzyl 2-

[[bis(benzyloxy)phosphoryl]methyl]pentanedioate using

Scheme III

10 Dibenzyl phosphite (9.5 g, 36 mmol) in 350 ml of dichloromethane was cooled to 0° C. To this stirring solution was added trimethyl aluminum (18.2 ml, 2.0 M solution in hexane, 36.4 mmol). After 30 minutes, dibenzyl 2-methylenepentanedioate (2) (6.0 g, 37 mmol) in
15 90 ml of dichloromethane was added dropwise over 10 minutes. The clear and colorless solution was then warmed to room temperature and left to stir overnight. The mixture was then quenched by the slow addition of 5% HCl. After stirring an additional 1.5 hours the lower
20 organic layer was removed and the aqueous layer extracted once with 100 ml of dichloromethane. The organics were combined, dried (MgSO₄), and evaporated to give a clear light golden liquid. The liquid was chromatographed on silica gel (4cm*30cm) and eluted with a gradient (4:1-
25 1:1) solvent system (Hexane/EtOAc). The fractions containing the desired product were combined and

WO 98/13044

PCT/US97/14417

158

evaporated to yield dibenzyl 2-[[bis(benzyloxy)-phosphoryl]methyl]pentanedioate (7.1 g, 42%) as a clear and colorless liquid. The liquid was then distilled on a Kughleror apparatus at 0.5 mm Hg and 195-200° C. The distillate was discarded and the remaining light golden oil was chromatographed on silica gel (1:1, Hexane/EtOAc) to give 2.9 g of dibenzyl 2-[[bis(benzyloxy)phosphoryl]methyl]pentanedioate as a clear and colorless oil. TLC R_f 0.5 (1:1 Hexane/EtOAc).

^1H NMR (CDCl_3): 7.1-7.4 (m, 20H), 5.05 (s, 2H), 4.8-5.03 (m, 6H), 2.8 (1H), 2.22-2.40 (m, 3H), 1.80-2.02 (m, 3H).

EXAMPLE 14

Preparation of 2-(phosphonomethyl)pentanedioic acid

15 (Compound 3) using Scheme III

Benzyl pentanedioate 2 (2.9 g, 4.9 mmol) was added to a mixture of 20 ml of methanol containing 0.29 g (6 mol %) of 10% Pd/C. This mixture was hydrogenated at 40 psi for 24 hours, filtered and evaporated to give 3 (1.0 g, 90%) as a clear slightly golden viscous oil.

^1H NMR (D_2O): 2.6-2.78 (m, 1H), 2.25-2.40 (m, 2H), 1.75-2.15 (m, 4H).

EXAMPLE 15

25 A patient is at risk of injury from an ischemic event. The patient may be pretreated with an effective

WO 98/13044

PCT/US97/14417

159

amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the pretreatment, the patient would be protected from any injury due to the ischemic event.

5

EXAMPLE 16

A patient is suffering from an ischemic event. The patient may be administered during or after the event, an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover or would not suffer any significant injury due to the ischemic event.

10

EXAMPLE 17

A patient has suffered injury from an ischemic event. The patient may be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would recover from the injury due to the ischemic event.

EXAMPLE 18

A patient is suffering from a glutamate abnormality. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition

15

25

WO 98/13044

PCT/US97/14417

160

of the present invention. It is expected that after the treatment, the patient would be protected from further injury due to the glutamate abnormality or would recover from the glutamate abnormality.

5

EXAMPLE 19

A patient is suffering from or has suffered from a nervous insult, such as that arising from a neurodegenerative disease or a neurodegenerative process.

10 The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further injury due to the nervous insult or would recover from
15 the nervous insult.

EXAMPLE 20

A patient is suffering from Parkinson's disease. The patient may then be administered an effective amount
20 of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from Parkinson's disease.

25

WO 98/13044

PCT/US97/14417

161

EXAMPLE 21

A patient is suffering from ALS. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from ALS.

EXAMPLE 22

A patient is suffering from epilepsy. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from epilepsy.

EXAMPLE 23

A patient is suffering from abnormalities in myelination/demyelination processes. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further neurodegeneration or would recover from the abnormalities in myelination/demyelination processes.

WO 98/13044

PCT/US97/14417

162

EXAMPLE 24

A patient is suffering from or has suffered from a cerebrovascular accident, such as stroke. The patient may then be administered an effective amount of a
5 NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any injury due to the cerebrovascular accident.

10

EXAMPLE 25

A patient is suffering from a head trauma. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of
15 the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury resulting from the head trauma.

20

EXAMPLE 26

A patient is suffering from a spinal trauma. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the
25 treatment, the patient would be protected from or would recover from any ischemic injury resulting from the

WO 98/13044

PCT/US97/14417

163

spinal trauma.

EXAMPLE 27

A patient is about to undergo surgery. The patient
5 may be administered an effective amount of a NAALADase
inhibitor or a pharmaceutical composition of the present
invention. It is expected that after the treatment, the
patient would not develop any ischemic brain, spinal or
peripheral injury resulting from or associated with the
10 surgery.

EXAMPLE 28

A patient is suffering from focal ischemia, such as
that associated with thromboembolytic occlusion of a
15 cerebral vessel, traumatic head injury, edema or brain
tumors. The patient may then be administered an
effective amount of a NAALADase inhibitor or a
pharmaceutical composition of the present invention. It
is expected that after the treatment, the patient would
20 be protected from or would recover from any brain, spinal
or peripheral injury resulting from the focal ischemia.

EXAMPLE 29

A patient is suffering from global ischemia. The
25 patient may then be administered an effective amount of
a NAALADase inhibitor or a pharmaceutical composition of

WO 98/13044

PCT/US97/14417

164

the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any brain, spinal or peripheral injury resulting from the global ischemia.

5

EXAMPLE 30

A patient is suffering from a cardiac arrest. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the cardiac arrest.

15

EXAMPLE 31

A patient is suffering from hypoxia, asphyxia or perinatal asphyxia. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic brain, spinal or peripheral injury associated with the hypoxia, asphyxia or perinatal asphyxia.

25

EXAMPLE 32

A patient is suffering from a cerebro-cortical

WO 98/13044

PCT/US97/14417

165

injury. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would
5 be protected from or would recover from any ischemic brain injury resulting from the cerebro-cortical injury.

EXAMPLE 33

The patient is suffering from an injury to the
10 caudate nucleus. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from or would recover from any ischemic
15 brain injury resulting from the injury to the caudate nucleus.

EXAMPLE 34

A patient is suffering from a cortical injury due to
20 a condition identified in these examples. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further
25 injury, or would exhibit at least 65% to at least 80% recovery from the cortical injury.

WO 98/13044

PCT/US97/14417

166

EXAMPLE 35

A patient is suffering from multiple sclerosis. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further demyelination or would recover from multiple sclerosis.

EXAMPLE 36

10 A patient is suffering from a peripheral neuropathy caused by Guillain-Barré syndrome. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient would be protected from further demyelination or would recover from the peripheral neuropathy.

EXAMPLE 37

20 The patient is suffering from alcoholism. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for alcohol would be suppressed.

25

WO 98/13044

PCT/US97/14417

167

EXAMPLE 38

A patient is suffering from nicotine dependence. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition
5 of the present invention. It is expected that after the treatment, the patient's craving for nicotine would be suppressed.

EXAMPLE 39

10 The patient is suffering from cocaine dependence. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for cocaine would be
15 suppressed.

EXAMPLE 40

A patient is suffering from heroine dependence. The patient may then be administered an effective amount of
20 a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's craving for heroine would be suppressed.

25

EXAMPLE 41

The patient is suffering from compulsive overeating,

WO 98/13044

PCT/US97/14417

168

obesity or severe obesity. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's
5 compulsion to eat would be suppressed.

EXAMPLE 42

A patient is suffering from pathological gambling. The patient may then be administered an effective amount
10 of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the patient's compulsion to gamble would be suppressed.

15

EXAMPLE 43

The patient is suffering from ADD. The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention. It is expected that after the treatment, the
20 patient's symptoms of inattention, impulsivity and/or hyperactivity would be suppressed.

EXAMPLE 44

A patient is suffering from Tourette's syndrome.
25 The patient may then be administered an effective amount of a NAALADase inhibitor or a pharmaceutical composition

WO 98/13044

PCT/US97/14417

169

of the present invention. It is expected that after the treatment, the patient's simple, complex, respiratory and vocal tics would be suppressed.

5

EXAMPLE 45

A patient is diagnosed with a disease, disorder or condition as identified in these examples. An effective amount of a NAALADase inhibitor or a pharmaceutical composition of the present invention may then be administered to the patient intravenously, intramuscularly, intraventricularly to the brain, rectally, subcutaneously, intranasally, through a catheter with or without a pump, orally, through a transdermal patch, topically, or through a polymer implant. After the treatment, the patient's condition would be expected to improve.

EXAMPLE 46

A patient is diagnosed with a disease, disorder or condition as identified in these examples. A NAALADase inhibitor or a pharmaceutical composition of the present invention may then be administered to the patient in the form of a 100 mg/kg bolus, optionally followed by a 20 mg/kg per hour intravenous infusion over a two-hour period. After the treatment, the patient's condition would be expected to improve.

WO 98/13044

PCT/US97/14417

170

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such
5 modifications are intended to be included within the scope of the following claims.

WO 98/13044

PCT/US97/14417

171

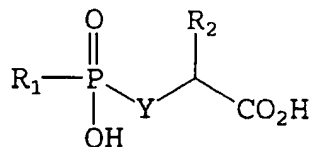
WE CLAIM:

1. A pharmaceutical composition comprising:
 - (i) an effective amount of a NAALADase inhibitor
5 for treating a compulsive disorder; and
 - (ii) a pharmaceutically acceptable carrier.
2. The pharmaceutical composition of claim 1,
further comprising at least one additional therapeutic
10 agent.
3. The pharmaceutical composition of claim 1,
wherein said NAALADase inhibitor is selected from the
group consisting of a glutamate-derived hydroxyphosphinyl
15 derivative, an acidic peptide analog, a conformationally
restricted glutamate mimic and mixtures thereof.
4. The pharmaceutical composition of claim 3,
wherein the NAALADase inhibitor is an acidic peptide
20 analog selected from the group consisting of Asp-Glu,
Glu-Glu, Gly-Glu, gamma-Glu-Glu and Glu-Glu-Glu.
5. The pharmaceutical composition of claim 3,
wherein the NAALADase inhibitor is a glutamate-derived
25 hydroxyphosphinyl derivative of formula I:

WO 98/13044

PCT/US97/14417

172



I

5

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

Y is CR₃R₄, NR₅ or O;

R₁ and R₅ are independently selected from the group
10 consisting of hydrogen, C₁-C₉ straight or branched chain
alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈
cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is
unsubstituted or substituted with carboxy, C₃-C₈
cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro,
15 trifluoromethyl, C₁-C₆ straight or branched chain alkyl,
C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-
C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture
thereof;

R₂ is selected from the group consisting of
20 hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉
straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
C₇ cycloalkenyl and Ar, wherein said R₂ is unsubstituted
or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
25 C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy,

WO 98/13044

PCT/US97/14417

173

phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₃ and R₄ are independently selected from the group consisting of hydrogen, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, Ar, halo and mixtures thereof;

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

6. The pharmaceutical composition of claim 5, wherein Y is CH₂.

7. The pharmaceutical composition of claim 6, wherein R₂ is substituted with carboxy.

8. The pharmaceutical composition of claim 7, wherein:

R₁ is hydrogen, C₁-C₄ straight or branched chain alkyl, C₂-C₄ straight or branched chain alkenyl, C₃-C₈

WO 98/13044

PCT/US97/14417

174

cycloalkyl, C₅-C₇ cycloalkenyl, benzyl or phenyl, wherein said R₁ is unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, benzyl, phenyl or a mixture thereof; and

R₂ is C₁-C₂ alkyl.

9. The pharmaceutical composition of claim 8, wherein the glutamate-derived hydroxyphosphinyl derivative is selected from the group consisting of:

2-(phosphonomethyl)pentanedioic acid;

2-(phosphonomethyl)succinic acid;

2-[[(2-carboxyethyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[(benzylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[(phenylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[[(hydroxy)phenylmethyl]hydroxyphosphinyl]methyl]pentanedioic acid;

2-[(butylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(3-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic acid;

2-[(3-phenylpropylhydroxyphosphinyl)methyl]pentanedioic acid;

2-[[(4-fluorophenyl)hydroxyphosphinyl]methyl]pentanedioic

WO 98/13044

PCT/US97/14417

175

- acid;
- 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid;
- 2-[(phenylethylhydroxyphosphinyl)methyl]pentanedioic acid;
- 5 2-[[(4-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic acid;
- 2-[[(4-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic acid;
- 2-[[(4-methoxybenzyl)hydroxyphosphinyl]methyl]pentane-
- 10 dioic acid;
- 2-[[(3-trifluoromethylbenzyl)hydroxyphosphinyl]methyl] - pentanedioic acid;
- 2-[[(2-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic acid;
- 15 2-[[(pentafluorobenzyl)hydroxyphosphinyl]methyl]pentane- dioic acid;
- 2-[[(phenylprop-2-enyl)hydroxyphosphinyl]methyl]pentane- dioic acid;
- 2-[[(aminomethyl)hydroxyphosphinyl]methyl]pentanedioic
- 20 acid;
- 2-[[(aminoethyl)hydroxyphosphinyl]methyl]pentanedioic acid;
- 2-[[(aminopropyl)hydroxyphosphinyl]methyl]pentanedioic acid; and
- 25 pharmaceutically acceptable salts and hydrates thereof.

WO 98/13044

PCT/US97/14417

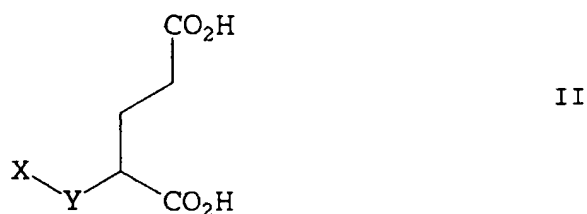
176

10. The pharmaceutical composition of claim 9, wherein the glutamate-derived hydroxyphosphinyl derivative is 2-(phosphonomethyl)pentanedioic acid or a pharmaceutically acceptable salt or hydrate thereof.

5

11. The pharmaceutical composition of claim 1, wherein the NAALADase inhibitor is a compound of formula II:

10

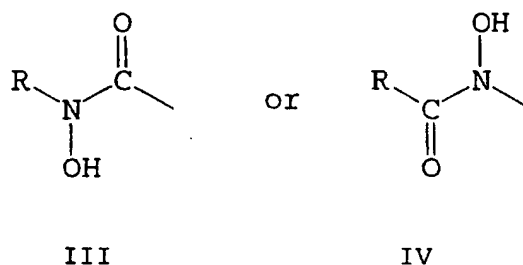


or a pharmaceutically acceptable salt or hydrate thereof,

15 wherein:

X is

20

Y is CR₁R₂, NR₃ or O;

25

R, R₁, R₂ and R₃ are independently selected from the group consisting of hydrogen, C₁-C₈ straight or branched

WO 98/13044

PCT/US97/14417

177

chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, Ar and mixtures thereof, wherein said R, R₁, R₂ and R₃ are independently unsubstituted or substituted with C₁-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

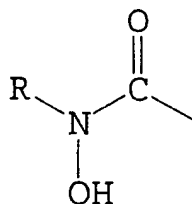
12. The pharmaceutical composition of claim 11, wherein Y is CH₂.

13. The pharmaceutical composition of claim 12, wherein X is

WO 98/13044

PCT/US97/14417

178



III

5

14. The pharmaceutical composition of claim 13, wherein R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-pyridyl, benzyl and phenyl, said R having one to three substituent(s) independently selected from the group consisting of hydrogen, C₁-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

15. The pharmaceutical composition of claim 14, wherein the compound is selected from the group consisting of:

2-[[(N-hydroxy) carbamoyl]methyl]pentanedioic acid;

2-[[(N-hydroxy-N-methyl) carbamoyl]methyl]pentanedioic acid;

2-[[(N-butyl-N-hydroxy) carbamoyl]methyl]pentanedioic acid;

WO 98/13044

PCT/US97/14417

179

2- [[(N-benzyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy-N-phenyl) carbamoyl] methyl] pentanedioic acid;

5 2- [[(N-hydroxy-N-2-phenylethyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-ethyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

10 2- [[(N-hydroxy-N-propyl) carbamoyl] methyl] pentanedioic acid;

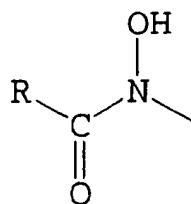
2- [[(N-hydroxy-N-3-phenylpropyl) carbamoyl] methyl] pentanedioic acid; and

2- [[(N-hydroxy-N-4-pyridyl) carbamoyl] methyl] pentanedioic acid.

15

16. The pharmaceutical composition of claim 12, wherein X is

20



IV

17. The pharmaceutical composition of claim 16,
25 wherein R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-

WO 98/13044

PCT/US97/14417

180

pyridyl, benzyl and phenyl, said R having one to three substituent(s) independently selected from the group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₇ alkoxy, C₂-C₇ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

18. The pharmaceutical composition of claim 17, wherein the compound is selected from the group consisting of:

2-[[[N-hydroxy)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(methyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(benzyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(phenyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(2-phenylethyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(ethyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(propyl)carboxamido)methyl]pentanedioic acid;

2-[[N-hydroxy(3-phenylpropyl)carboxamido)methyl]pentanedioic acid; and

2-[[N-hydroxy(4-pyridyl)carboxamido)methyl]pentanedioic

WO 98/13044

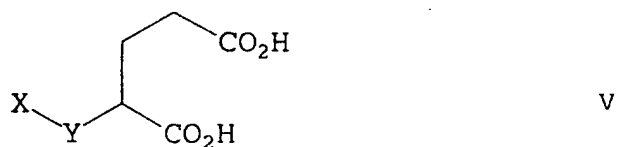
PCT/US97/14417

181

acid.

19. The pharmaceutical composition of claim 1,
wherein the NAALADase inhibitor is a compound of formula

5 V:

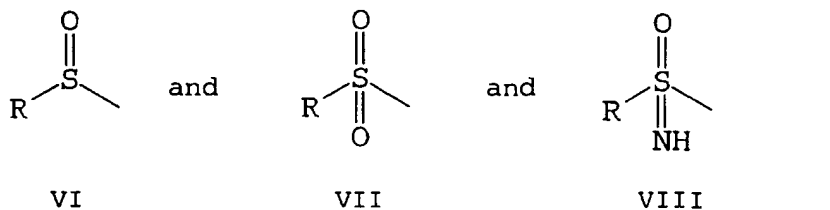


10

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

X is selected from the group consisting of

15



20

Y is CR₁R₂, NR₃ or O;

R, R₁, R₂ and R₃ are independently selected from the
group consisting of hydrogen, C₁-C₈ straight or branched
chain alkyl, C₂-C₈ straight or branched chain alkenyl, C₃-
C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R,
25 R₁, R₂ and R₃ are independently unsubstituted or
substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl,

WO 98/13044

PCT/US97/14417

182

halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

5 Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, said Ar having one to three substituent(s) independently selected from the group
10 consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino and mixtures thereof.

15

20. The pharmaceutical composition of claim 19, wherein in said compound, at least one of said R, R₁, R₂ and R₃ is/are independently substituted with C₁-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, hydroxy, halo, nitro,
20 trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof.

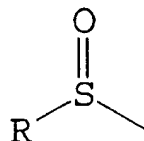
25 21. The pharmaceutical composition of claim 20, wherein in said compound, Y is CH₂.

WO 98/13044

PCT/US97/14417

183

22. The pharmaceutical composition of claim 21,
wherein in said compound, X is



23. The pharmaceutical composition of claim 22,
wherein in said compound, R is selected from the group
10 consisting of hydrogen, C₁-C₄ straight or branched chain
alkyl, 4-pyridyl, benzyl and phenyl, said R having one to
three substituent(s) independently selected from the
group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
15 C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy,
phenoxy, benzyloxy, amino, Ar and mixtures thereof.

24. The pharmaceutical composition of claim 23,
20 wherein said compound is selected from the group
consisting of:

2-[(sulfinyl)methyl]pentanedioic acid;

2-[(methylsulfinyl)methyl]pentanedioic acid;

2-[(ethylsulfinyl)methyl]pentanedioic acid;

25 2-[(propylsulfinyl)methyl]pentanedioic acid;

2-[(butylsulfinyl)methyl]pentanedioic acid;

WO 98/13044

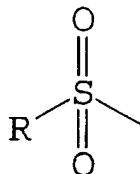
PCT/US97/14417

184

- 2-[(phenylsulfinyl)methyl]pentanedioic acid;
2-[[(2-phenylethyl)sulfinyl]methyl]pentanedioic acid;
2-[[(3-phenylpropyl)sulfinyl]methyl]pentanedioic acid;
2-[[(4-pyridyl)sulfinyl]methyl]pentanedioic acid; and
5 2-[(benzylsulfinyl)methyl]pentanedioic acid.

25. The pharmaceutical composition of claim 24,
wherein in said compound, X is

10



VII

26. The pharmaceutical composition of claim 25,
15 wherein in said compound, R is selected from the group
consisting of hydrogen, C₁-C₄ straight or branched chain
alkyl, 4-pyridyl, benzyl and phenyl, said R having one to
three substituent(s) independently selected from the
group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇
20 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy,
phenoxy, benzyloxy, amino, Ar and mixtures thereof.

- 25 27. The pharmaceutical composition of claim 26,
wherein said compound is selected from the group

WO 98/13044

PCT/US97/14417

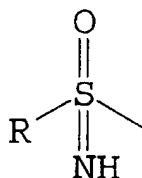
185

consisting of:

- 2-[(sulfonyl)methyl]pentanedioic acid;
- 2-[(methylsulfonyl)methyl]pentanedioic acid;
- 2-[(ethylsulfonyl)methyl]pentanedioic acid;
- 5 2-[(propylsulfonyl)methyl]pentanedioic acid;
- 2-[(butylsulfonyl)methyl]pentanedioic acid;
- 2-[(phenylsulfonyl)methyl]pentanedioic acid;
- 2-[[(2-phenylethyl) sulfonyl]methyl]pentanedioic acid;
- 2-[[(3-phenylpropyl) sulfonyl]methyl]pentanedioic acid;
- 10 2-[[(4-pyridyl) sulfonyl]methyl]pentanedioic acid; and
- 2-[(benzylsulfonyl)methyl]pentanedioic acid.

28. The pharmaceutical composition of claim 27,
wherein in said compound, X is

15



VIII

- 20 29. The pharmaceutical composition of claim 28,
wherein in said compound, R is selected from the group
consisting of hydrogen, C₁-C₄ straight or branched chain
alkyl, 4-pyridyl, benzyl and phenyl, said R having one to
three substituent(s) independently selected from the
25 group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-

WO 98/13044

PCT/US97/14417

186

C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

5 30. The pharmaceutical composition of claim 29, wherein said compound is selected from the group consisting of:

2-[(sulfoximinyl)methyl]pentanedioic acid;

2-[(methylsulfoximinyl)methyl]pentanedioic acid;

10 2-[(ethylsulfoximinyl)methyl]pentanedioic acid;

2-[(propylsulfoximinyl)methyl]pentanedioic acid;

2-[(butylsulfoximinyl)methyl]pentanedioic acid;

2-[(phenylsulfoximinyl)methyl]pentanedioic acid;

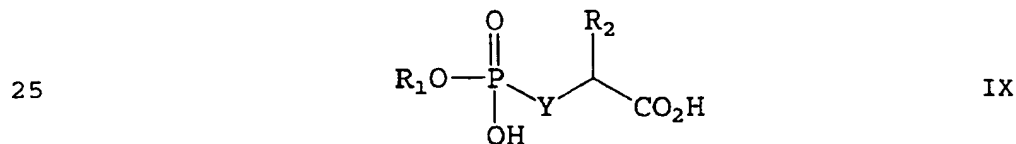
2-[[[(2-phenylethyl)sulfoximinyl]methyl]pentanedioic acid;

15 2-[[[(3-phenylpropyl)sulfoximinyl]methyl]pentanedioic acid;

2-[[[(4-pyridyl)sulfoximinyl]methyl]pentanedioic acid; and

2-[(benzylsulfoximinyl)methyl]pentanedioic acid.

20 31. The pharmaceutical composition of claim 1, wherein the NAALADase inhibitor is a compound of formula IX:



WO 98/13044

PCT/US97/14417

187

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

Y is CR₃R₄, NR₅ or O;

R₂ is selected from the group consisting of
5 hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉
straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-
C₇ cycloalkenyl and Ar, wherein said R₂ is unsubstituted
or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
10 C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy,
phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₁, R₃, R₄ and R₅ are independently selected from the
group consisting of hydrogen, C₁-C₉ straight or branched
15 chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-
C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R,
R₁, R₂ and R₃ are independently unsubstituted or
substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl,
halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or
20 branched chain alkyl, C₂-C₆ straight or branched chain
alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy,
benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-
naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-
25 furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-
pyridyl, benzyl and phenyl, wherein said Ar has one to

WO 98/13044

PCT/US97/14417

188

three substituent(s) independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-
5 C₆ alkenyloxy, phenoxy, benzyloxy, amino and mixtures thereof.

32. The pharmaceutical composition of claim 31, wherein Y is CH₂.

10

33. The pharmaceutical composition of claim 32, wherein R is hydrogen.

34. The pharmaceutical composition of claim 33,
15 wherein the compound is selected from the group consisting of:

phosphonopropanoic acid;

2-methyl-3-phosphonopropanoic acid;

2-ethyl-3-phosphonopropanoic acid;

20 2-propyl-3-phosphonopropanoic acid;

2-butyl-3-phosphonopropanoic acid;

2-phenyl-3-phosphonopropanoic acid;

2-(2-phenylethyl)-3-phosphonopropanoic acid;

2-(3-phenylpropyl)-3-phosphonopropanoic acid;

25 2-(4-pyridyl)-3-phosphonopropanoic acid; and

2-benzyl-3-phosphonopropanoic acid.

WO 98/13044

PCT/US97/14417

189

35. The pharmaceutical composition of claim 32, wherein R₂ is substituted with carboxy.

36. The pharmaceutical composition of claim 35, wherein the compound is selected from the group consisting of:

2- (hydrohydroxyphosphonomethyl)pentanedioic acid;

2- (hydromethoxyphosphonomethyl)pentanedioic acid;

2- (hydroethoxyphosphonomethyl)pentanedioic acid;

10 2- (hydropropoxyphosphonomethyl)pentanedioic acid;

2- (hydrobutoxyphosphonomethyl)pentanedioic acid;

2- (hydrophenoxyphosphonomethyl)pentanedioic acid;

2- [hydro (2-phenylethoxy) phosphonomethyl]pentanedioic acid;

15 2- [hydro (3-phenylpropoxy) phosphonomethyl]pentanedioic acid;

2- [hydro (4-pyridyloxy) phosphonomethyl]pentanedioic acid;

and

2- (hydrobenzyloxyphosphonomethyl)pentanedioic acid.

20

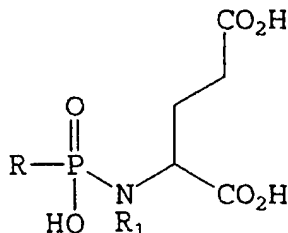
37. The pharmaceutical composition of claim 1, wherein the NAALADase inhibitor is a compound of formula X:

WO 98/13044

PCT/US97/14417

190

5



X

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

R and R₁ are independently selected from the group
10 consisting of hydrogen, C₁-C₆ straight or branched chain
alkyl or alkenyl group, C₃-C₈ cycloalkyl, C₃ or C₅
cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R and
R₁ are independently unsubstituted or substituted with
C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy,
15 nitro, trifluoromethyl, C₁-C₆ straight or branched chain
alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆
alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar
or a mixture thereof; and

Ar is selected from the group consisting of 1-
20 naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-
furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-
thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl,
benzyl and phenyl, wherein said Ar is unsubstituted or
substituted with halo, hydroxy, nitro, trifluoromethyl,
25 C₁-C₆ straight or branched alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy,

WO 98/13044

PCT/US97/14417

191

phenoxy, benzyloxy, amino or a mixture thereof.

38. The pharmaceutical composition of claim 37,
wherein the compound is selected from the group
5 consisting of:

N-[methylhydroxyphosphinyl]glutamic acid;

N-[ethylhydroxyphosphinyl]glutamic acid;

N-[propylhydroxyphosphinyl]glutamic acid;

N-[butylhydroxyphosphinyl]glutamic acid;

10 N-[phenylhydroxyphosphinyl]glutamic acid;

N-[(phenylmethyl)hydroxyphosphinyl]glutamic acid;

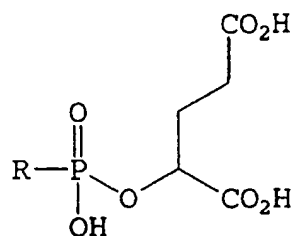
N-[(2-phenylethyl)methyl]hydroxyphosphinyl]glutamic
acid; and

N-methyl-N-[phenylhydroxyphosphinyl]glutamic acid.

15

39. The pharmaceutical composition of claim 1,
wherein the NAALADase inhibitor is a compound of formula
XI:

20



XI

25

WO 98/13044

PCT/US97/14417

192

or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

R is selected from the group consisting of hydrogen,
C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or
5 branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, Ar and mixtures thereof, wherein said R is
unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
C₆ straight or branched chain alkyl, C₂-C₆ straight or
10 branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy,
phenoxy, benzyloxy, amino, Ar or a mixture thereof;

Ar is selected from the group consisting of 1-
naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-
furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or
15 phenyl, having one to three substituents which are
independently selected from the group consisting of
hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C₁-C₆
straight or branched alkyl or alkenyl, C₁-C₆ alkoxy or C₁-
C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

20

40. The pharmaceutical composition of claim 39,
wherein the compound is selected from the group
consisting of:

2-[[methylhydroxyphosphinyl]oxy]pentanedioic acid;
25 2-[[ethylhydroxyphosphinyl]oxy]pentanedioic acid;
2-[[propylhydroxyphosphinyl]oxy]pentanedioic acid;

WO 98/13044

PCT/US97/14417

193

- 2- [[butylhydroxyphosphinyl]oxy]pentanedioic acid;
2- [[phenylhydroxyphosphinyl]oxy]pentanedioic acid;
2- [[[(4-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic
acid;
5 2- [[[(2-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic
acid;
2- [[(phenylmethyl)hydroxyphosphinyl]oxy]pentanedioic
acid; and
2- [[[(2-phenylethyl)methyl]hydroxyphosphinyl]oxy] -
10 pentanedioic acid.

41. A method of treating a compulsive disorder,
comprising administering an effective amount of a
NAALADase inhibitor to a patient in need thereof.
15

42. The method of claim 41, wherein the NAALADase
inhibitor is administered in combination with at least
one additional therapeutic agent.

20 43. The method of claim 41, wherein said NAALADase
inhibitor is selected from the group consisting of a
glutamate-derived hydroxyphosphinyl derivative, an acidic
peptide analog, a conformationally restricted glutamate
mimic and mixtures thereof.

25

WO 98/13044

PCT/US97/14417

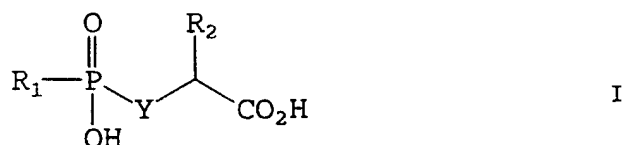
194

44. The method of claim 43, wherein the NAALADase inhibitor is an acidic peptide analog selected from the group consisting of Asp-Glu, Glu-Glu, Gly-Glu, gamma-Glu-Glu and Glu-Glu-Glu.

5

45. The method of claim 43, wherein the NAALADase inhibitor is a glutamate-derived hydroxyphosphinyl derivative of formula I:

10



or a pharmaceutically acceptable salt or hydrate thereof,

15 wherein:

Y is CR₃R₄, NR₅ or O;

R₁ and R₅ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁ is unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof;

20
25

WO 98/13044

PCT/US97/14417

195

R_2 is selected from the group consisting of hydrogen, C_1 - C_8 straight or branched chain alkyl, C_2 - C_8 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl and Ar, wherein said R_2 is unsubstituted
5 or substituted with carboxy, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof;

10 R_3 and R_4 are independently selected from the group consisting of hydrogen, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, Ar, halo and mixtures thereof;

15 Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or
20 substituted with halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

25 46. The method of claim 45, wherein Y is CH_2 .

WO 98/13044

PCT/US97/14417

196

47. The method of claim 46, wherein R_2 is substituted with carboxy.

48. The method of claim 47, wherein:

5 R_1 is hydrogen, C_1-C_4 straight or branched chain alkyl, C_2-C_4 straight or branched chain alkenyl, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, benzyl or phenyl, wherein said R_1 is unsubstituted or substituted with carboxy, C_3-C_8 cycloalkyl, C_5-C_7 cycloalkenyl, halo, hydroxy, nitro,
10 trifluoromethyl, C_1-C_6 straight or branched chain alkyl, C_2-C_6 straight or branched chain alkenyl, C_1-C_4 alkoxy, C_2-C_4 alkenyloxy, phenoxy, benzyloxy, amino, benzyl, phenyl or a mixture thereof; and

R_2 is C_1-C_2 alkyl.

15

49. The method of claim 48, wherein the glutamate-derived hydroxyphosphinyl derivative is selected from the group consisting of:

2- (phosphonomethyl)pentanedioic acid;

20 2- (phosphonomethyl)succinic acid;

2- [(2-carboxyethyl)hydroxyphosphinyl)methyl]pentanedioic acid;

2- [(benzylhydroxyphosphinyl)methyl]pentanedioic acid;

2- [(phenylhydroxyphosphinyl)methyl]pentanedioic acid;

25 2- [[((hydroxy)phenylmethyl)hydroxyphosphinyl)methyl] - pentanedioic acid;

WO 98/13044

PCT/US97/14417

197

- 2-[(butylhydroxyphosphinyl)methyl]pentanedioic acid;
2-[[(3-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2-[(3-phenylpropylhydroxyphosphinyl)methyl]pentanedioic
5 acid;
2-[[(4-fluorophenyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid;
2-[(phenylethylhydroxyphosphinyl)methyl]pentanedioic
10 acid;
2-[[(4-methylbenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
2-[[(4-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic
acid;
15 2-[[(4-methoxybenzyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(3-trifluoromethylbenzyl)hydroxyphosphinyl]methyl]-
pentanedioic acid;
2-[[(2-fluorobenzyl)hydroxyphosphinyl]methyl]pentanedioic
20 acid;
2-[[(pentafluorobenzyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
2-[[(phenylprop-2-enyl)hydroxyphosphinyl]methyl]pentane-
dioic acid;
25 2-[[(aminomethyl)hydroxyphosphinyl]methyl]pentanedioic
acid;

WO 98/13044

PCT/US97/14417

198

2-[[(aminoethyl)hydroxyphosphinyl]methyl]pentanedioic acid;

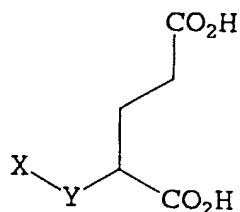
2-[[(aminopropyl)hydroxyphosphinyl]methyl]pentanedioic acid; and

5 pharmaceutically acceptable salts and hydrates thereof.

50. The method of claim 49, wherein the glutamate-derived hydroxyphosphinyl derivative is 2-(phosphonomethyl)pentanedioic acid or a pharmaceutically
10 acceptable salt or hydrate thereof.

51. The method of claim 41, wherein the NAALADase inhibitor is a compound of formula II:

15

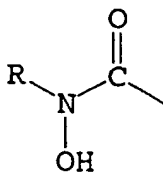


II

or a pharmaceutically acceptable salt or hydrate thereof,

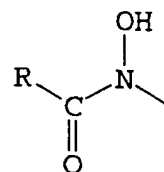
20 wherein:

X is



III

or



IV

25

WO 98/13044

PCT/US97/14417

199

Y is CR_1R_2 , NR_3 or O;

R, R_1 , R_2 and R_3 are independently selected from the group consisting of hydrogen, C_1 - C_9 straight or branched chain alkyl, C_2 - C_9 straight or branched chain alkenyl, C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, Ar and mixtures thereof, wherein said R, R_1 , R_2 and R_3 are independently unsubstituted or substituted with C_3 - C_8 cycloalkyl, C_5 - C_7 cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_9 alkoxy, C_2 - C_9 alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C_1 - C_6 straight or branched chain alkyl, C_2 - C_6 straight or branched chain alkenyl, C_1 - C_6 alkoxy, C_2 - C_6 alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

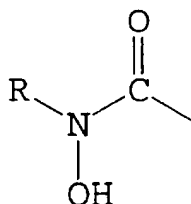
52. The method of claim 51, wherein Y is CH_2 .

53. The method of claim 52, wherein X is

WO 98/13044

PCT/US97/14417

200



III

5

54. The pharmaceutical composition of claim 53, wherein R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-pyridyl, benzyl and phenyl, said R having one to three substituent(s) independently selected from the group consisting of hydrogen, C₁-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

55. The method of claim 54, wherein the compound is selected from the group consisting of:

2- [[(N-hydroxy) carbamoyl] methyl] pentanedioic acid;

2- [[(N-hydroxy-N-methyl) carbamoyl] methyl] pentanedioic acid;

2- [[(N-butyl-N-hydroxy) carbamoyl] methyl] pentanedioic acid;

2- [[(N-benzyl-N-hydroxy) carbamoyl] methyl] pentanedioic

WO 98/13044

PCT/US97/14417

201

acid;

2- [[(N-hydroxy-N-phenyl) carbamoyl] methyl] pentanedioic
acid;

2- [[(N-hydroxy-N-2-phenylethyl) carbamoyl] methyl] pentane-
5 dioic acid;

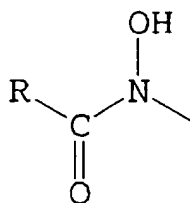
2- [[(N-ethyl-N-hydroxy) carbamoyl] methyl] pentanedioic
acid;

2- [[(N-hydroxy-N-propyl) carbamoyl] methyl] pentanedioic
acid;

10 2- [[(N-hydroxy-N-3-phenylpropyl) carbamoyl] methyl] pentane-
dioic acid; and

2- [[(N-hydroxy-N-4-pyridyl) carbamoyl] methyl] pentanedioic
acid.

15 56. The method of claim 52, wherein X is



IV

20

57. The method of claim 56, wherein R is selected
from the group consisting of hydrogen, C₁-C₄ straight or
branched chain alkyl, 4-pyridyl, benzyl and phenyl, said
25 R having one to three substituent(s) independently
selected from the group consisting of hydrogen, C₃-C₈

WO 98/13044

PCT/US97/14417

202

cycloalkyl, C₅-C₆ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures
5 thereof.

58. The method of claim 57, wherein the compound is selected from the group consisting of:

- 2-[[[N-hydroxy)carboxamido)methyl]pentanedioic acid;
10 2-[[[N-hydroxy(methyl)carboxamido)methyl]pentanedioic acid;
2-[[[N-hydroxy(benzyl)carboxamido)methyl]pentanedioic acid;
2-[[[N-hydroxy(phenyl)carboxamido)methyl]pentanedioic
15 acid;
2-[[[N-hydroxy(2-phenylethyl)carboxamido)methyl]pentanedioic acid;
2-[[[N-hydroxy(ethyl)carboxamido)methyl]pentanedioic acid;
2-[[[N-hydroxy(propyl)carboxamido)methyl]pentanedioic
20 acid;
2-[[[N-hydroxy(3-phenylpropyl)carboxamido)methyl]pentanedioic acid; and
2-[[[N-hydroxy(4-pyridyl)carboxamido)methyl]pentanedioic acid.

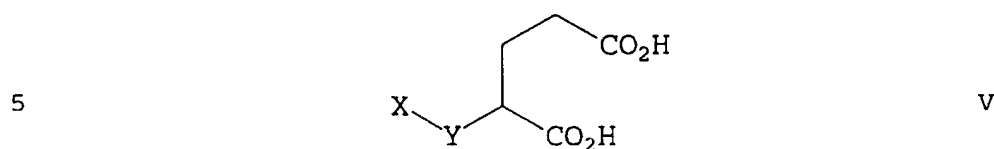
25

WO 98/13044

PCT/US97/14417

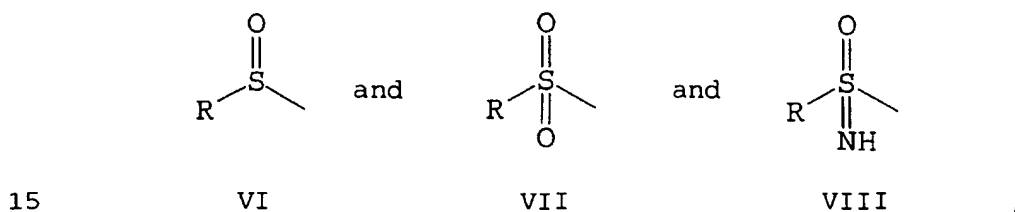
203

59. The method of claim 41, wherein the NAALADase inhibitor is a compound of formula V:



or a pharmaceutically acceptable salt or hydrate thereof,
wherein:

10 X is selected from the group consisting of



Y is CR₁R₂, NR₃ or O;

R, R₁, R₂ and R₃ are independently selected from the group consisting of hydrogen, C₁-C₈ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R, R₁, R₂ and R₃ are independently unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₈ alkoxy, C₂-C₉ alkenyloxy, phenoxy,

20

25

WO 98/13044

PCT/US97/14417

204

benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, said Ar having one to three
5 substituent(s) independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-
10 C₆ alkenyloxy, phenoxy, benzyloxy, amino and mixtures thereof.

60. The method of claim 59, wherein in said compound, at least one of said R, R₁, R₂ and R₃ is/are
15 independently substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, hydroxy, halo, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof.

20

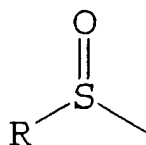
61. The method of claim 60, wherein in said compound, Y is CH₂.

25 62. The method of claim 61, wherein in said compound, X is

WO 98/13044

PCT/US97/14417

205



VI

5

63. The method of claim 62, wherein in said compound, R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-pyridyl, benzyl and phenyl, said R having one to three substituent(s) independently selected from the group consisting of hydrogen, C₁-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

64. The method of claim 63, wherein said compound is selected from the group consisting of:

20 2-[(sulfinyl)methyl]pentanedioic acid;
2-[(methylsulfinyl)methyl]pentanedioic acid;
2-[(ethylsulfinyl)methyl]pentanedioic acid;
2-[(propylsulfinyl)methyl]pentanedioic acid;
2-[(butylsulfinyl)methyl]pentanedioic acid;
25 2-[(phenylsulfinyl)methyl]pentanedioic acid;
2-[[(2-phenylethyl) sulfinyl]methyl]pentanedioic acid;

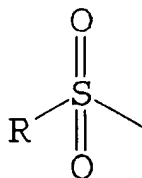
WO 98/13044

PCT/US97/14417

206

2-[[[(3-phenylpropyl)sulfinyl)methyl]pentanedioic acid;
2-[[[(4-pyridyl)sulfinyl)methyl]pentanedioic acid; and
2-[(benzylsulfinyl)methyl]pentanedioic acid.

5 65. The method of claim 64, wherein in said
compound, X is



VII

66. The method of claim 65, wherein in said
compound, R is selected from the group consisting of
hydrogen, C₁-C₄ straight or branched chain alkyl, 4-
15 pyridyl, benzyl and phenyl, said R having one to three
substituent(s) independently selected from the group
consisting of hydrogen, C₁-C₈ cycloalkyl, C₅-C₇,
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
C₆ straight or branched chain alkyl, C₂-C₆ straight or
20 branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy,
phenoxy, benzyloxy, amino, Ar and mixtures thereof.

67. The method of claim 66, wherein said compound
is selected from the group consisting of:
25 2-[(sulfonyl)methyl]pentanedioic acid;
2-[(methylsulfonyl)methyl]pentanedioic acid;

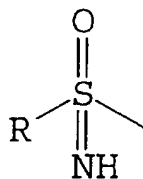
WO 98/13044

PCT/US97/14417

207

- 2-[(ethylsulfonyl)methyl]pentanedioic acid;
2-[(propylsulfonyl)methyl]pentanedioic acid;
2-[(butylsulfonyl)methyl]pentanedioic acid;
2-[(phenylsulfonyl)methyl]pentanedioic acid;
5 2-[[(2-phenylethyl)sulfonyl]methyl]pentanedioic acid;
2-[[(3-phenylpropyl)sulfonyl]methyl]pentanedioic acid;
2-[[(4-pyridyl)sulfonyl]methyl]pentanedioic acid; and
2-[(benzylsulfonyl)methyl]pentanedioic acid.

- 10 68. The method of claim 67, wherein in said compound, X is



VIII

15

69. The method of claim 68, wherein in said compound, R is selected from the group consisting of hydrogen, C₁-C₄ straight or branched chain alkyl, 4-pyridyl, benzyl and phenyl, said R having one to three
20 substituent(s) independently selected from the group consisting of hydrogen, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or
25 branched chain alkenyl, C₁-C₄ alkoxy, C₂-C₄ alkenyloxy, phenoxy, benzyloxy, amino, Ar and mixtures thereof.

WO 98/13044

PCT/US97/14417

208

70. The method of claim 69, wherein said compound is selected from the group consisting of:

2-[(sulfoximinyl)methyl]pentanedioic acid;

2-[(methylsulfoximinyl)methyl]pentanedioic acid;

5 2-[(ethylsulfoximinyl)methyl]pentanedioic acid;

2-[(propylsulfoximinyl)methyl]pentanedioic acid;

2-[(butylsulfoximinyl)methyl]pentanedioic acid;

2-[(phenylsulfoximinyl)methyl]pentanedioic acid;

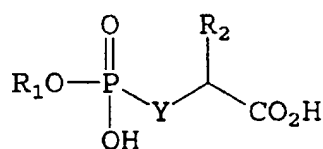
2-[[2-phenylethyl]sulfoximinyl]methyl]pentanedioic acid;

10 2-[[3-phenylpropyl]sulfoximinyl]methyl]pentanedioic acid;

2-[[4-pyridyl]sulfoximinyl]methyl]pentanedioic acid; and

2-[(benzylsulfoximinyl)methyl]pentanedioic acid.

15 71. The method of claim 41, wherein the NAALADase inhibitor is a compound of formula IX:



IX

20

or a pharmaceutically acceptable salt or hydrate thereof, wherein:

Y is CR₃R₄, NR₅ or O;

25 R₂ is selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉

WO 98/13044

PCT/US97/14417

209

straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₂ is unsubstituted or substituted with carboxy, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof;

R₁, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen, C₁-C₉ straight or branched chain alkyl, C₂-C₉ straight or branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R₁, R₂ and R₃ are independently unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar has one to three substituent(s) independently selected from the group consisting of hydrogen, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₉ alkoxy, C₂-C₉ alkenyloxy, phenoxy, benzyloxy, amino and mixtures

WO 98/13044

PCT/US97/14417

210

thereof.

72. The method of claim 71, wherein Y is CH₂.

5 73. The method of claim 72, wherein R is hydrogen.

74. The method of claim 73, wherein the compound is selected from the group consisting of:

phosphonopropanoic acid;

10 2-methyl-3-phosphonopropanoic acid;

2-ethyl-3-phosphonopropanoic acid;

2-propyl-3-phosphonopropanoic acid;

2-butyl-3-phosphonopropanoic acid;

2-phenyl-3-phosphonopropanoic acid;

15 2-(2-phenylethyl)-3-phosphonopropanoic acid;

2-(3-phenylpropyl)-3-phosphonopropanoic acid;

2-(4-pyridyl)-3-phosphonopropanoic acid; and

2-benzyl-3-phosphonopropanoic acid.

20 75. The method of claim 72, wherein R₂ is substituted with carboxy.

76. The method of claim 75, wherein the compound is selected from the group consisting of:

25 2-(hydroxyphosphonomethyl)pentanedioic acid;

2-(hydromethoxyphosphonomethyl)pentanedioic acid;

WO 98/13044

PCT/US97/14417

211

2- (hydroethoxyphosphonomethyl)pentanedioic acid;

2- (hydropropoxyphosphonomethyl)pentanedioic acid;

2- (hydrobutoxyphosphonomethyl)pentanedioic acid;

2- (hydrophenoxyphosphonomethyl)pentanedioic acid;

5 2- [hydro (2-phenylethoxy) phosphonomethyl] pentanedioic acid;

2- [hydro (3-phenylpropoxy) phosphonomethyl] pentanedioic acid;

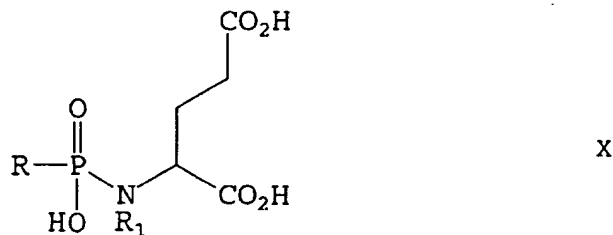
2- [hydro (4-pyridyloxy) phosphonomethyl] pentanedioic acid;

10 and

2- (hydrobenzyloxyphosphonomethyl)pentanedioic acid.

77. The method of claim 41, wherein the NAALADase inhibitor is a compound of formula X:

15



20

or a pharmaceutically acceptable salt or hydrate thereof, wherein:

R and R₁ are independently selected from the group
 25 consisting of hydrogen, C₁-C₈, straight or branched chain alkyl or alkenyl group, C₃-C₈, cycloalkyl, C₃, or C₅,

WO 98/13044

PCT/US97/14417

212

cycloalkyl, C₅-C₇ cycloalkenyl and Ar, wherein said R and R₁ are independently unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₇ cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched chain alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino, Ar or a mixture thereof; and

Ar is selected from the group consisting of 1-naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 4-indolyl, 2-furyl, 3-furyl, tetrahydrofuranyl, tetrahydropyranyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, benzyl and phenyl, wherein said Ar is unsubstituted or substituted with halo, hydroxy, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl, C₂-C₆ straight or branched chain alkenyl, C₁-C₆ alkoxy, C₂-C₆ alkenyloxy, phenoxy, benzyloxy, amino or a mixture thereof.

78. The method of claim 77, wherein the compound is selected from the group consisting of:

- N-[methylhydroxyphosphinyl]glutamic acid;
- N-[ethylhydroxyphosphinyl]glutamic acid;
- N-[propylhydroxyphosphinyl]glutamic acid;
- N-[butylhydroxyphosphinyl]glutamic acid;
- N-[phenylhydroxyphosphinyl]glutamic acid;
- N-[(phenylmethyl)hydroxyphosphinyl]glutamic acid;
- N-[(2-phenylethyl)methyl]hydroxyphosphinyl]glutamic

WO 98/13044

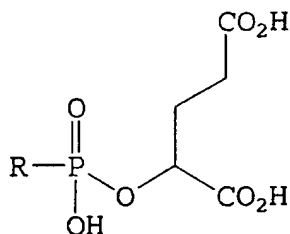
PCT/US97/14417

213

acid; and

N-methyl-N- [phenylhydroxyphosphinyl]glutamic acid.

79. The method of claim 41, wherein the NAALADase
5 inhibitor is a compound of formula XI:



XI

10

or a pharmaceutically acceptable salt or hydrate thereof,
15 wherein:

R is selected from the group consisting of hydrogen,
C₁-C₈ straight or branched chain alkyl, C₂-C₈ straight or
branched chain alkenyl, C₃-C₈ cycloalkyl, C₅-C₈
cycloalkenyl, Ar and mixtures thereof, wherein said R is
20 unsubstituted or substituted with C₃-C₈ cycloalkyl, C₅-C₈
cycloalkenyl, halo, hydroxy, nitro, trifluoromethyl, C₁-
C₆ straight or branched chain alkyl, C₂-C₆ straight or
branched chain alkenyl, C₁-C₈ alkoxy, C₂-C₈ alkenyloxy,
phenoxy, benzyloxy, amino, Ar or a mixture thereof;

25 Ar is selected from the group consisting of 1-
naphthyl, 2-naphthyl, 2-indolyl, 3-indolyl, 2-furyl, 3-

WO 98/13044

PCT/US97/14417

214

furyl, 2-thienyl, 3-thienyl, 2-, 3-, or 4-pyridyl, or phenyl, having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, trifluoromethyl, C₁-C₆ straight or branched alkyl or alkenyl, C₁-C₆ alkoxy or C₁-C₆ alkenyloxy, phenoxy, benzyloxy, and amino.

80. The method of claim 79, wherein the compound is selected from the group consisting of:

- 10 2-[[methylhydroxyphosphinyl]oxy]pentanedioic acid;
2-[[ethylhydroxyphosphinyl]oxy]pentanedioic acid;
2-[[propylhydroxyphosphinyl]oxy]pentanedioic acid;
2-[[butylhydroxyphosphinyl]oxy]pentanedioic acid;
2-[[phenylhydroxyphosphinyl]oxy]pentanedioic acid;
15 2-[[[(4-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic acid;
2-[[[(2-pyridyl)methyl]hydroxyphosphinyl]oxy]pentanedioic acid;
2-[[[(phenylmethyl)hydroxyphosphinyl]oxy]pentanedioic
20 acid; and
2-[[[(2-phenylethyl)methyl]hydroxyphosphinyl]oxy]-pentanedioic acid.

81. The method of claim 41, wherein the compulsive
25 disorder is selected from the group consisting of drug dependence, eating disorders, pathological gambling,

WO 98/13044

PCT/US97/14417

215

attention deficit disorder (ADD) and Tourette's syndrome.

82. The method of claim 81, wherein the drug dependence is alcohol dependence.

5

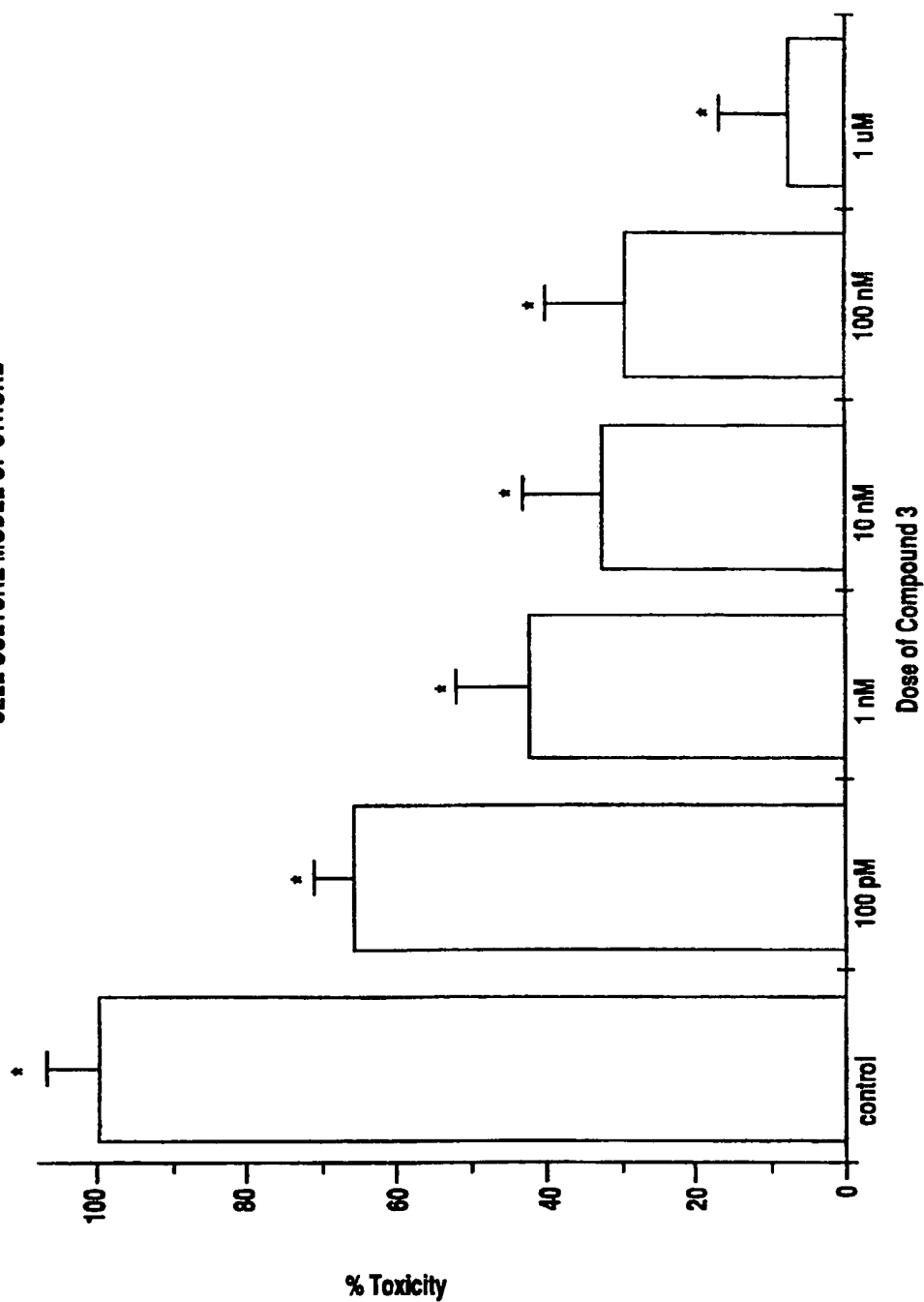
83. The method of claim 81, wherein the drug dependence is nicotine dependence.

WO 98/13044

PCT/US97/14417

1 / 17

FIG.1
COMPOUND 3 IS NEUROPROTECTIVE IN A
CELL CULTURE MODEL OF STROKE

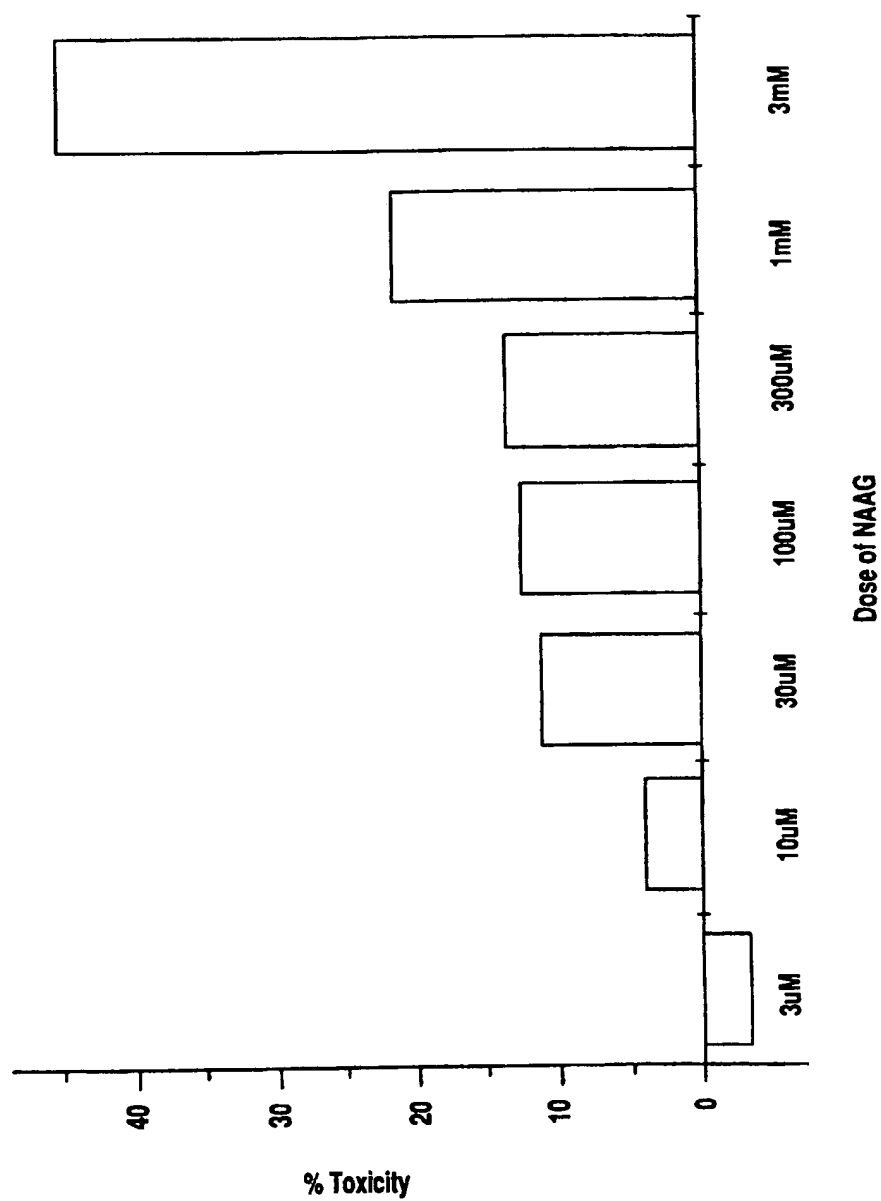


WO 98/13044

PCT/US97/14417

2 / 17

FIG.2
NAAG TOXICITY

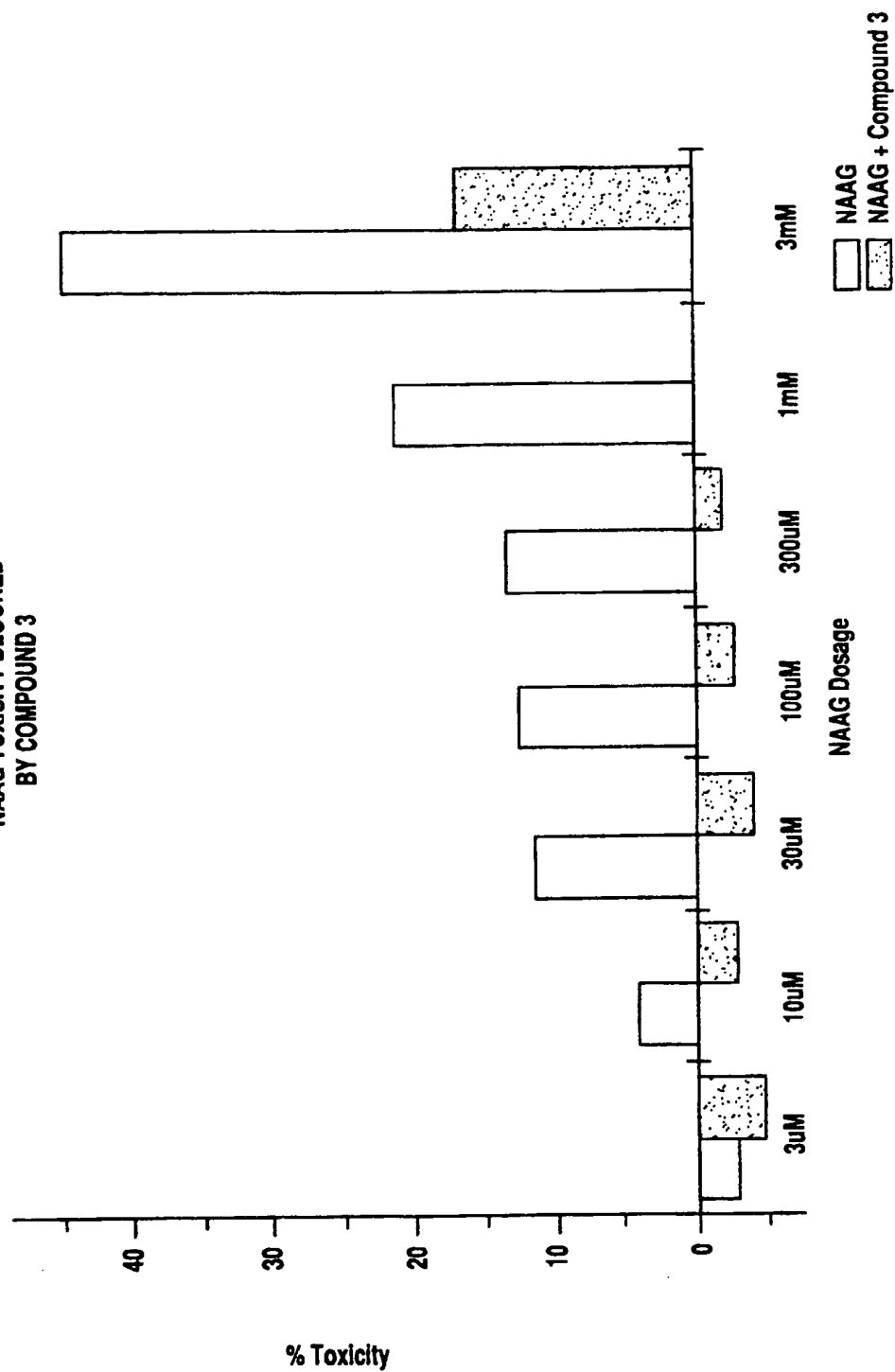


WO 98/13044

PCT/US97/14417

3 / 17

FIG.3

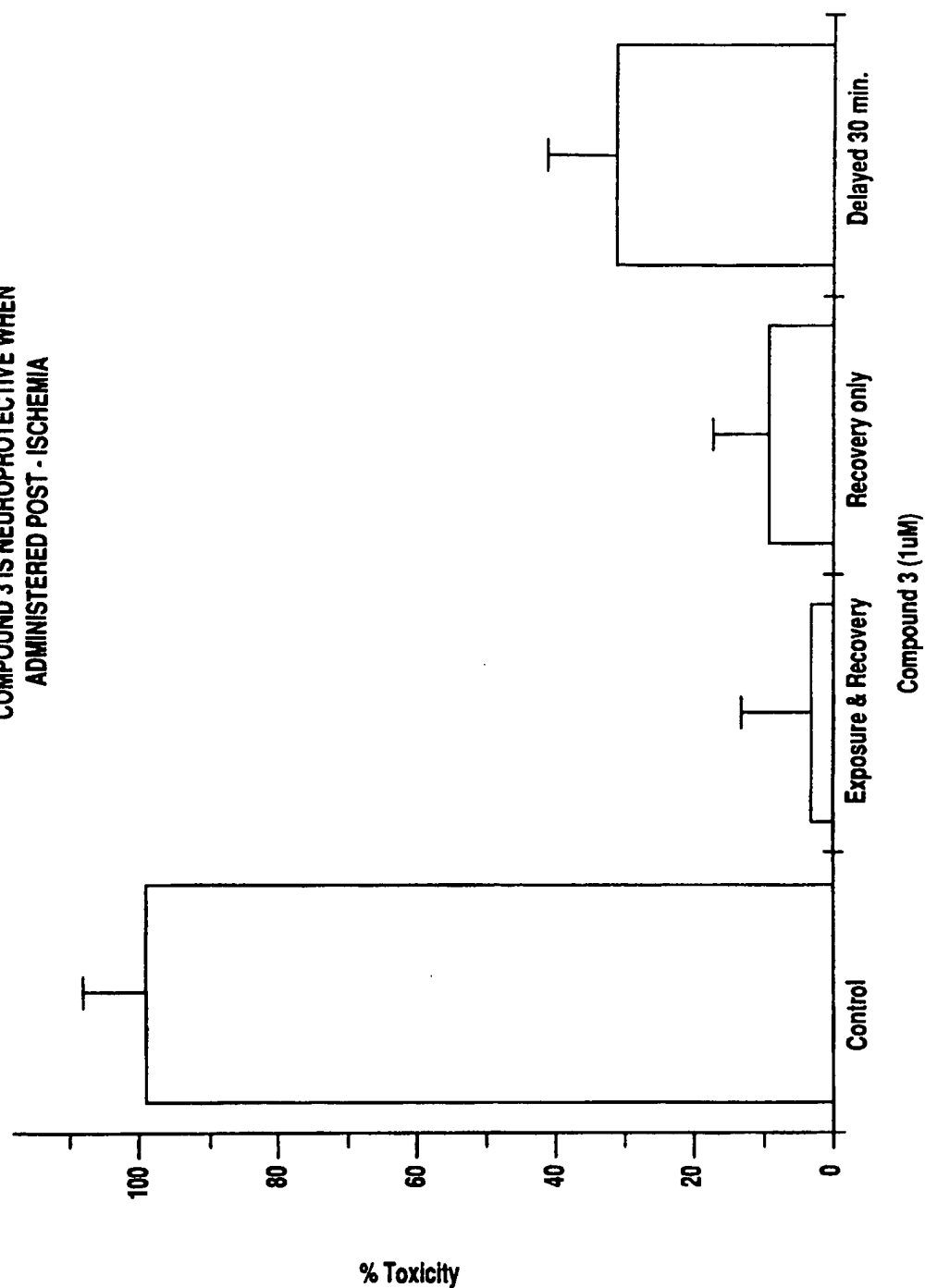
NAAG TOXICITY BLOCKED
BY COMPOUND 3

WO 98/13044

PCT/US97/14417

4 / 17

FIG.4
COMPOUND 3 IS NEUROPROTECTIVE WHEN
ADMINISTERED POST - ISCHEMIA

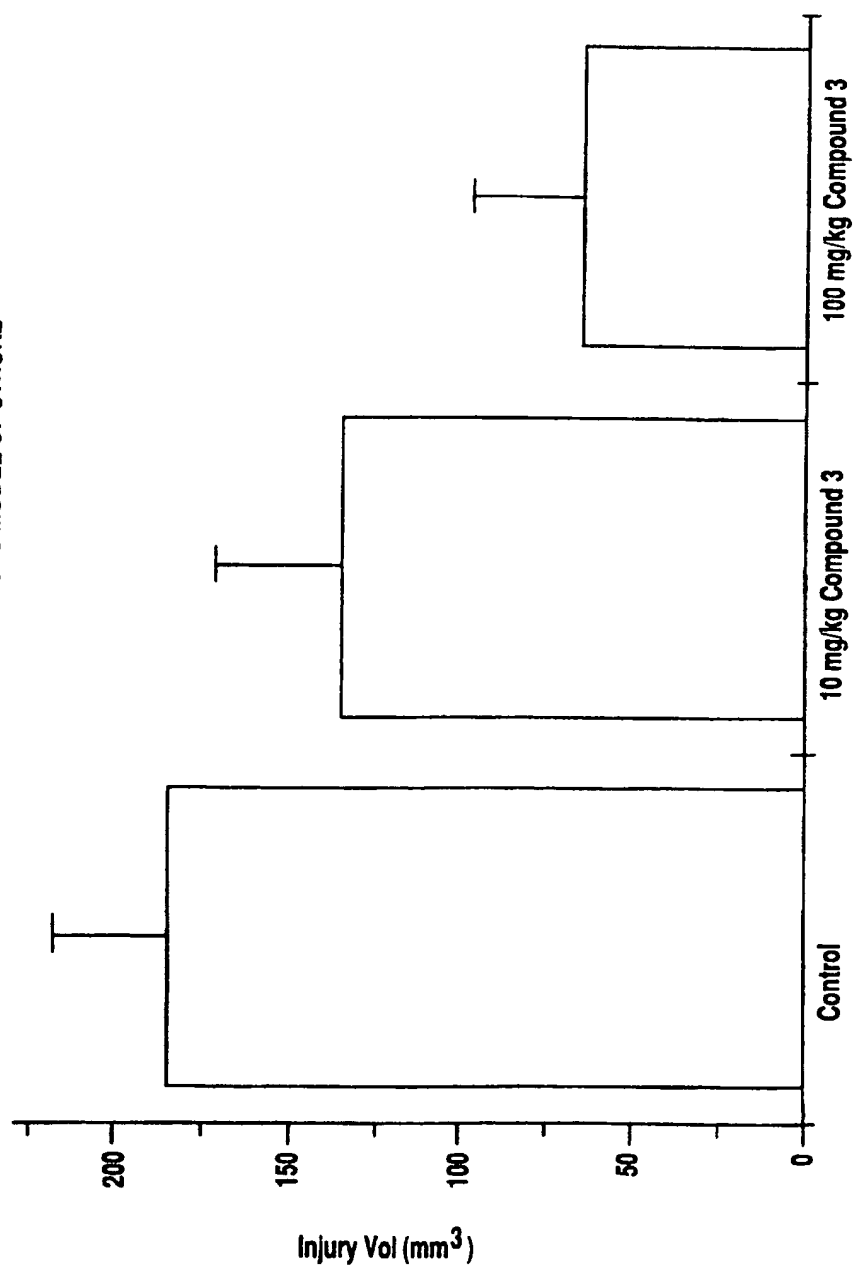


WO 98/13044

PCT/US97/14417

5 / 17

FIG.5
COMPOUND 3 IS NEUROPROTECTIVE
IN RAT MCAO MODEL OF STROKE



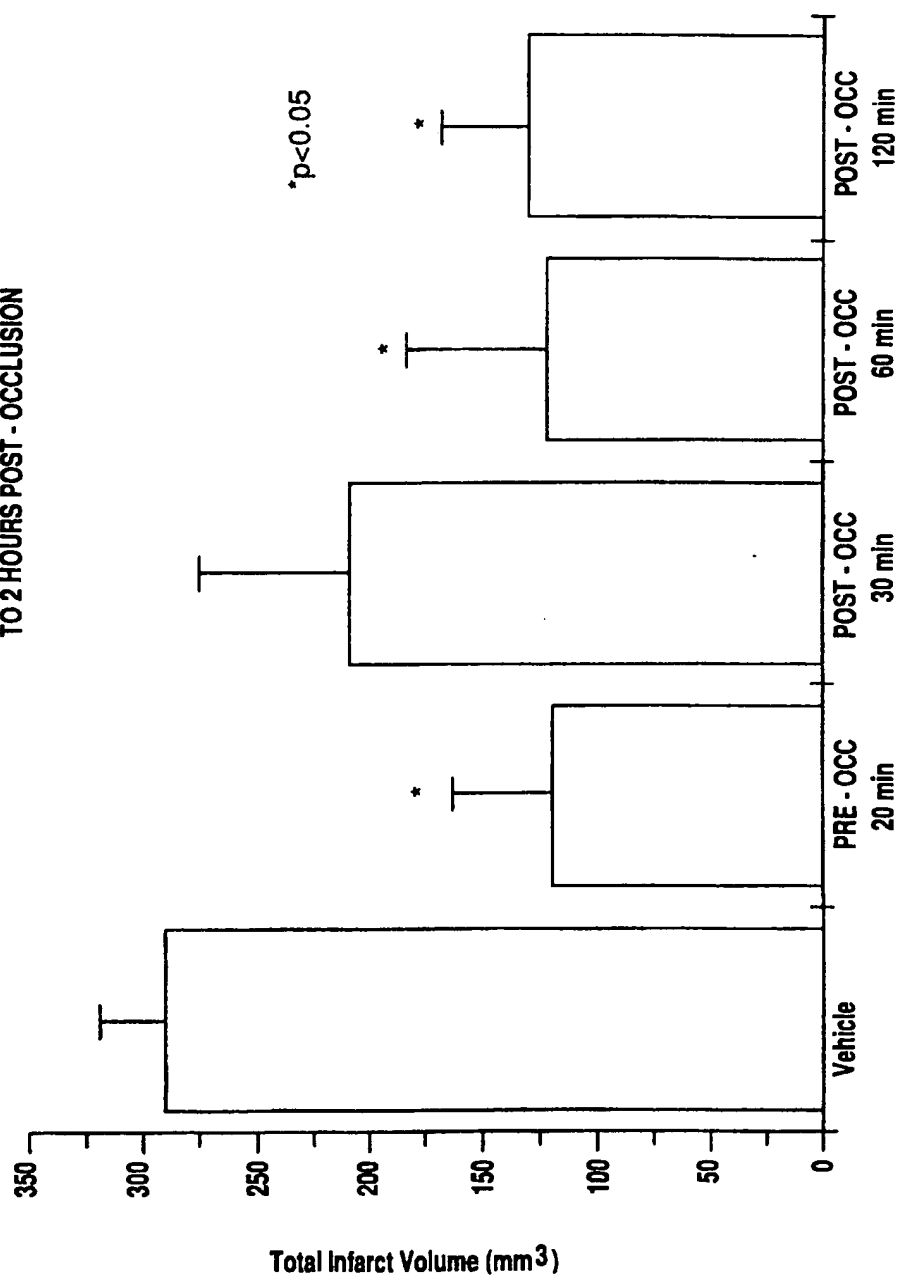
WO 98/13044

PCT/US97/14417

6 / 17

FIG.6

COMPOUND 3 IS NEUROPROTECTIVE IN RAT MCAO
MODEL OF STROKE WHEN ADMINISTERED UP
TO 2 HOURS POST - OCCLUSION

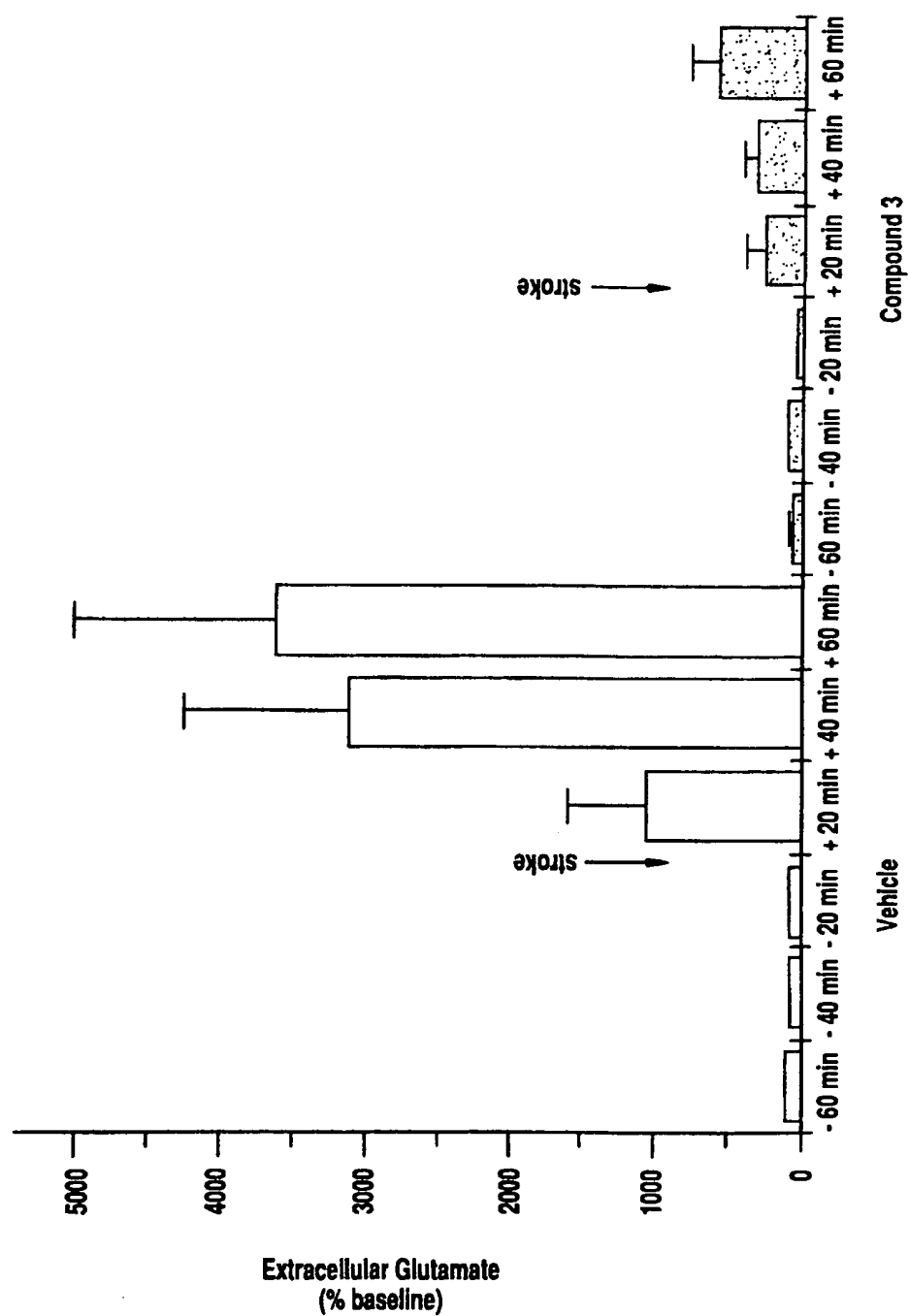


SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

7 / 17

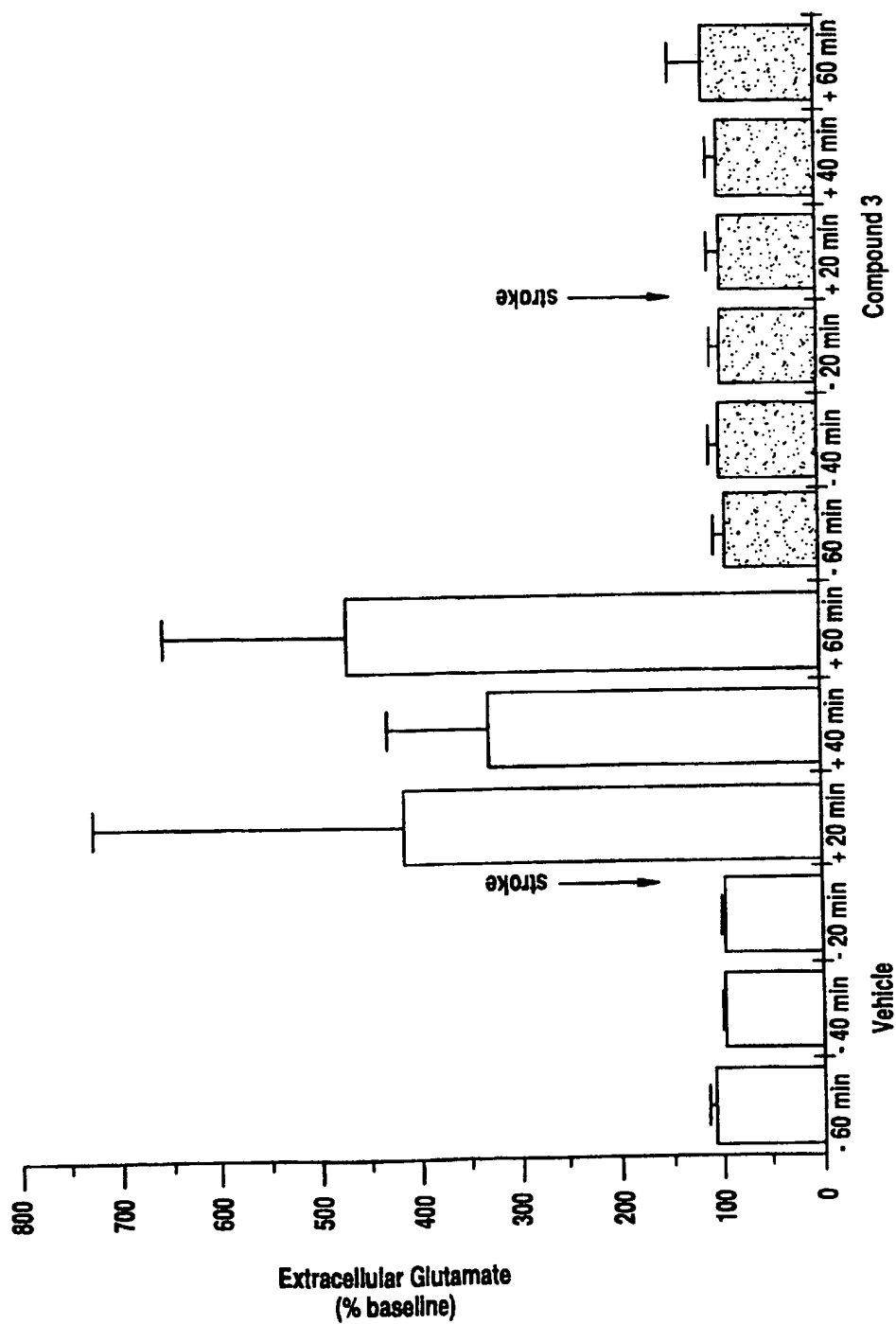
FIG.7
STRIATUM

WO 98/13044

PCT/US97/14417

8 / 17

FIG.8
PARIETAL CORTEX

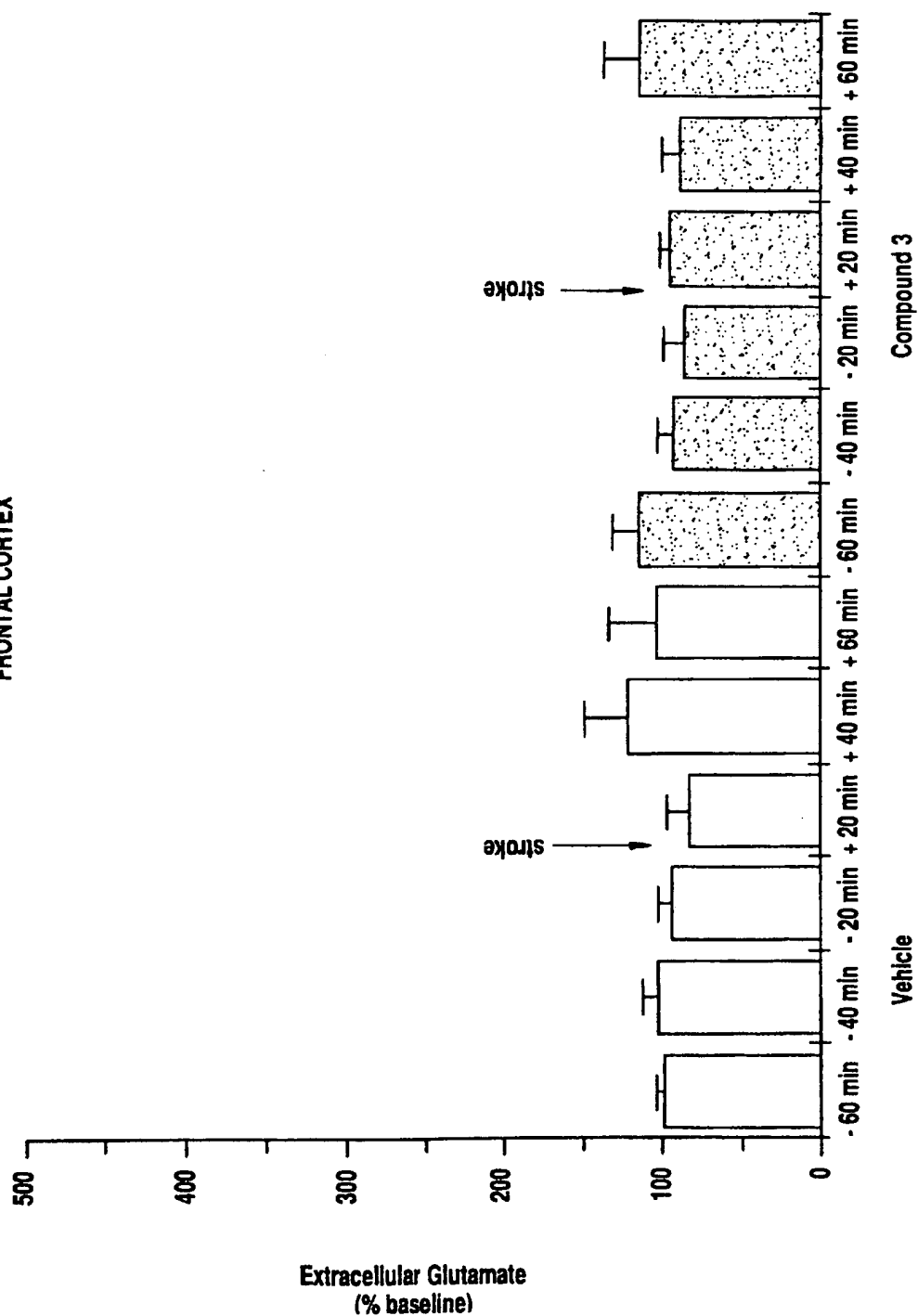


WO 98/13044

PCT/US97/14417

9 / 17

FIG. 9
FRONTAL CORTEX



SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

10 / 17

GPI 5000 Administration **Following Sciatic Nerve Crush**



GPI 5000 Polymer
2 ug drug / day

FIG.10B



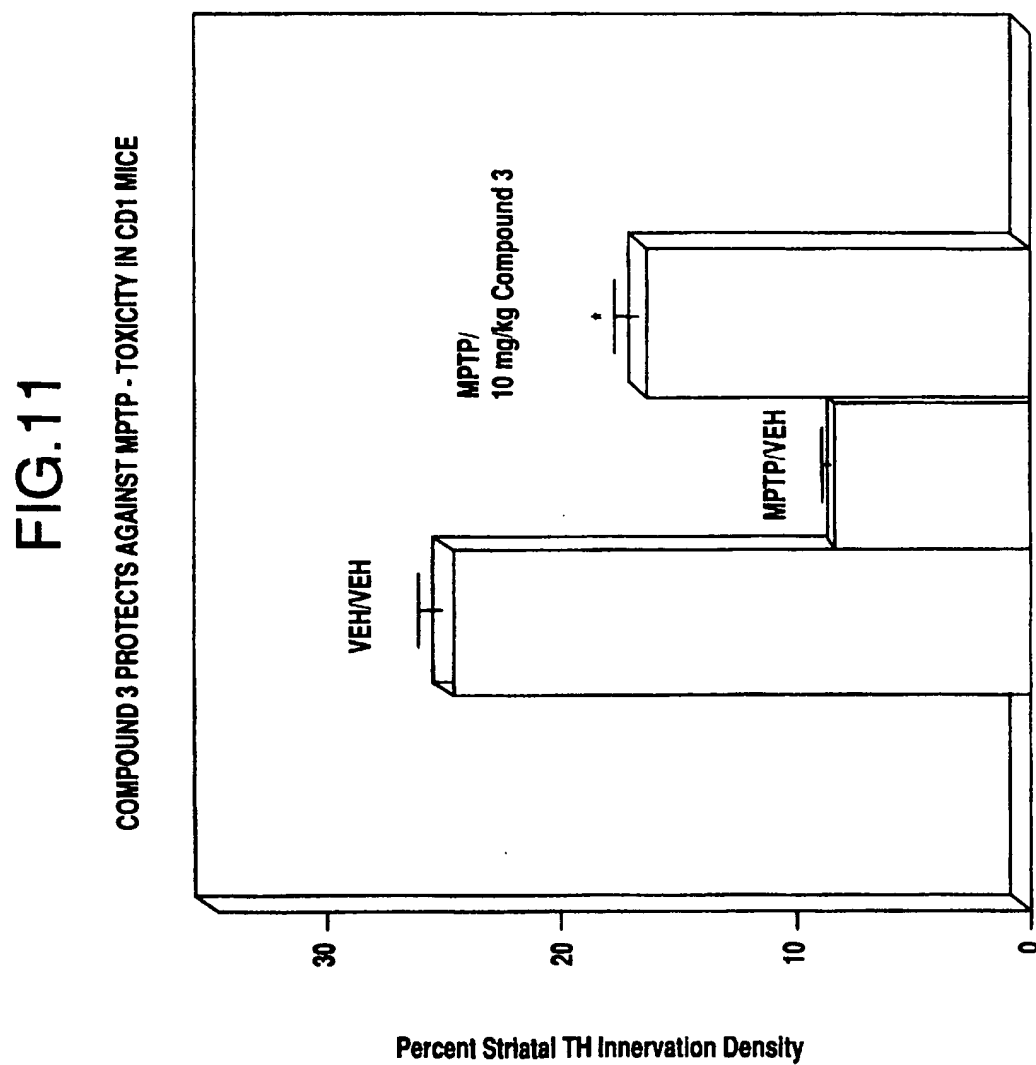
Vehicle Polymer

FIG.10A

WO 98/13044

PCT/US97/14417

11 / 17



SUBSTITUTE SHEET (RULE 26)

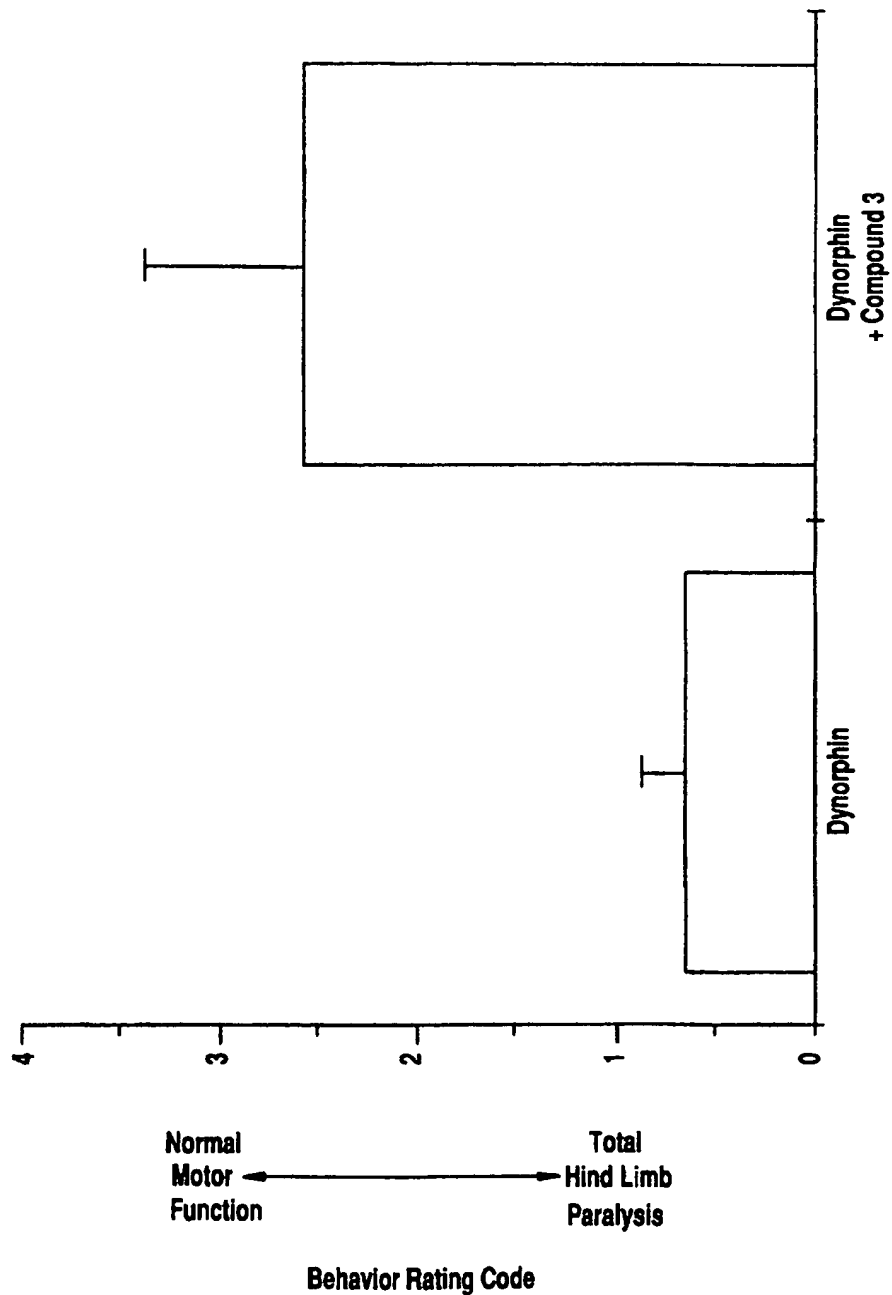
WO 98/13044

PCT/US97/14417

12 / 17

FIG.12

COMPOUND 3 IS NEUROPROTECTIVE IN A
DYNORPHIN - INDUCED SPINAL CORD INJURY MODEL

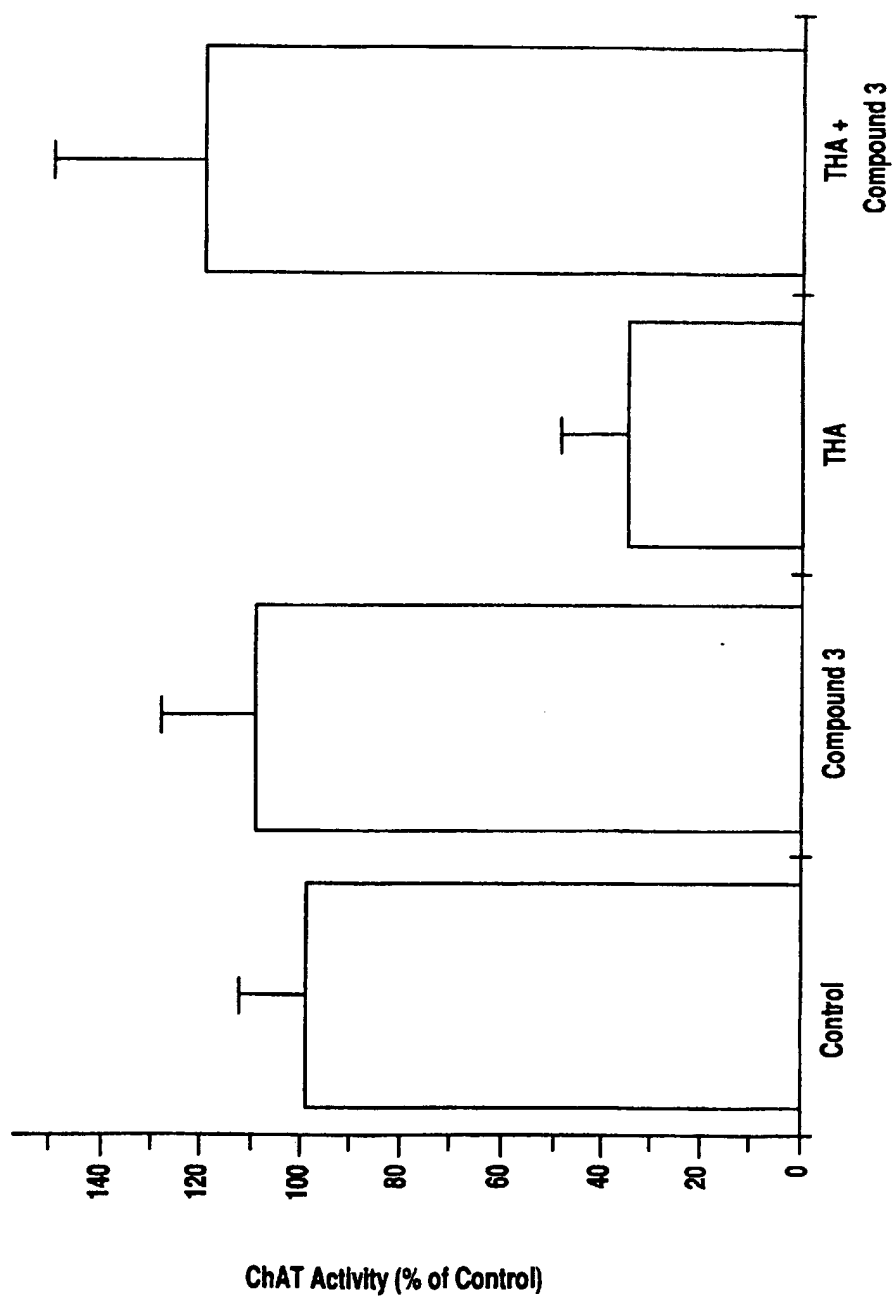


SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

13 / 12

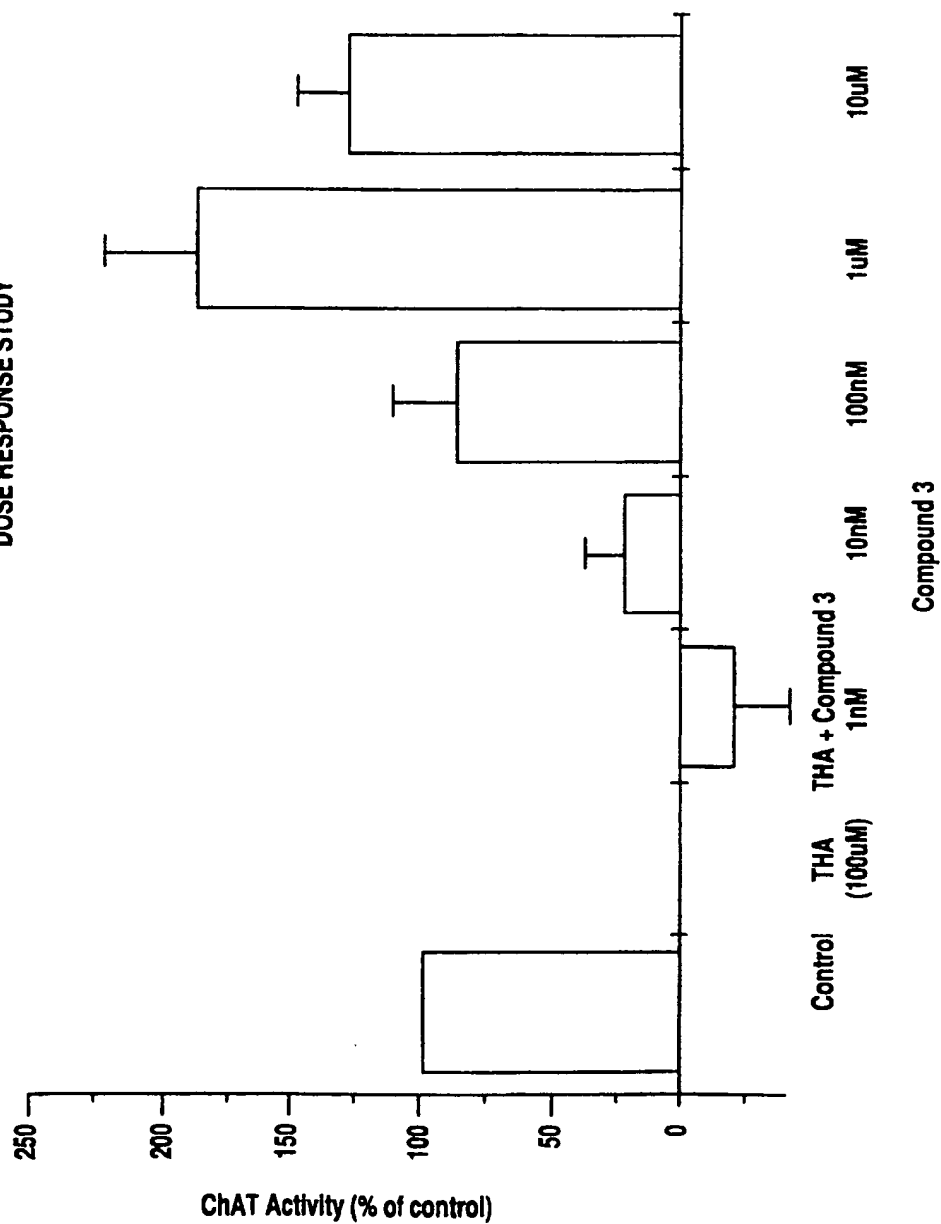
FIG.13**COMPOUND 3 IS NEUROPROTECTIVE IN A
SPINAL CORD CULTURE MODEL OF ALS**

WO 98/13044

PCT/US97/14417

14 / 17

FIG.14
COMPOUND 3 IS NEUROPROTECTIVE IN SPINAL CORD ORGANOTYPIC CULTURE MODEL OF ALS:
DOSE RESPONSE STUDY



SUBSTITUTE SHEET (RULE 26)

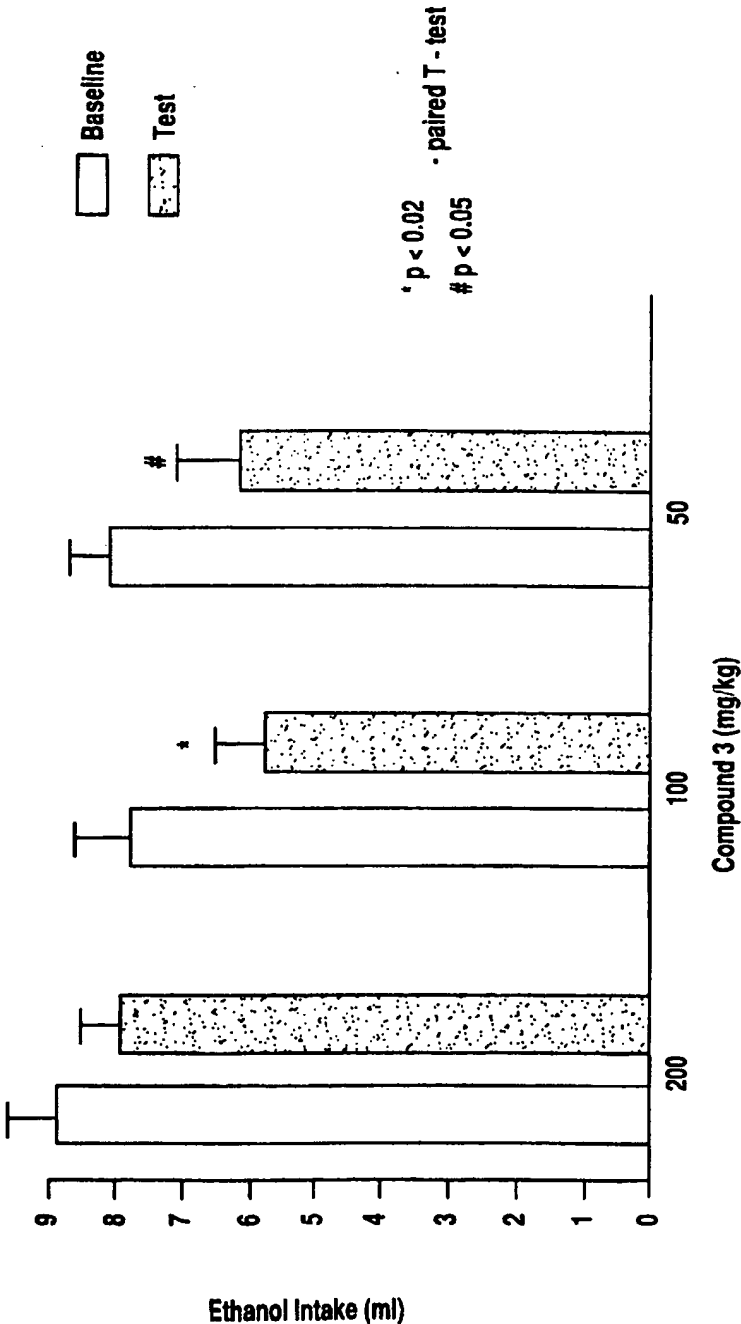
WO 98/13044

PCT/US97/14417

15 / 17

FIG.15

1 HR ETHANOL INTAKES



WO 98/13044

PCT/US97/14417

16 / 17

FIG. 16B

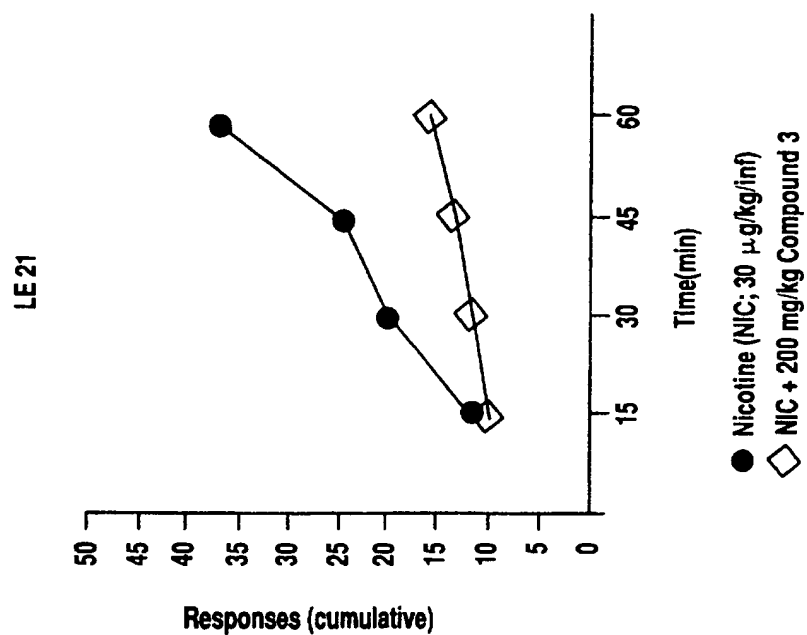
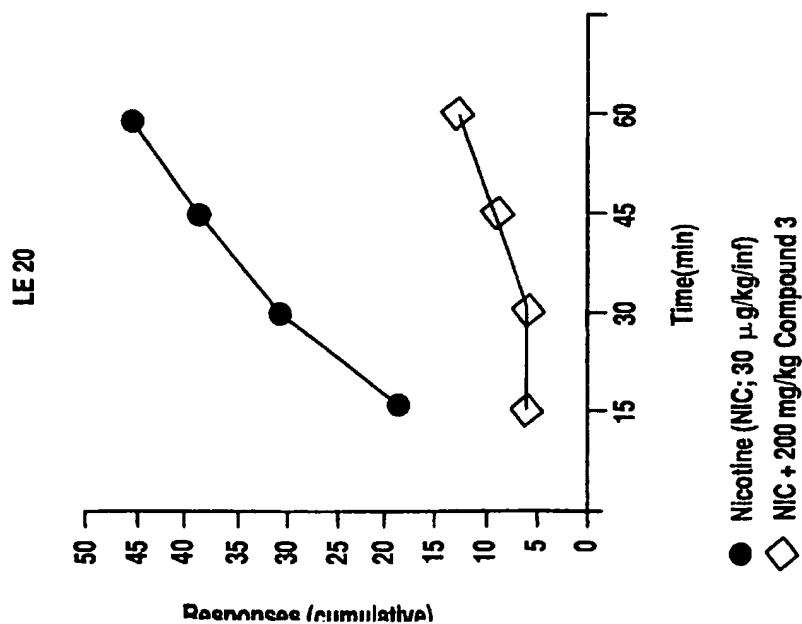


FIG. 16A



SUBSTITUTE SHEET (RULE 26)

WO 98/13044

PCT/US97/14417

17 / 17

FIG.17B

LE13

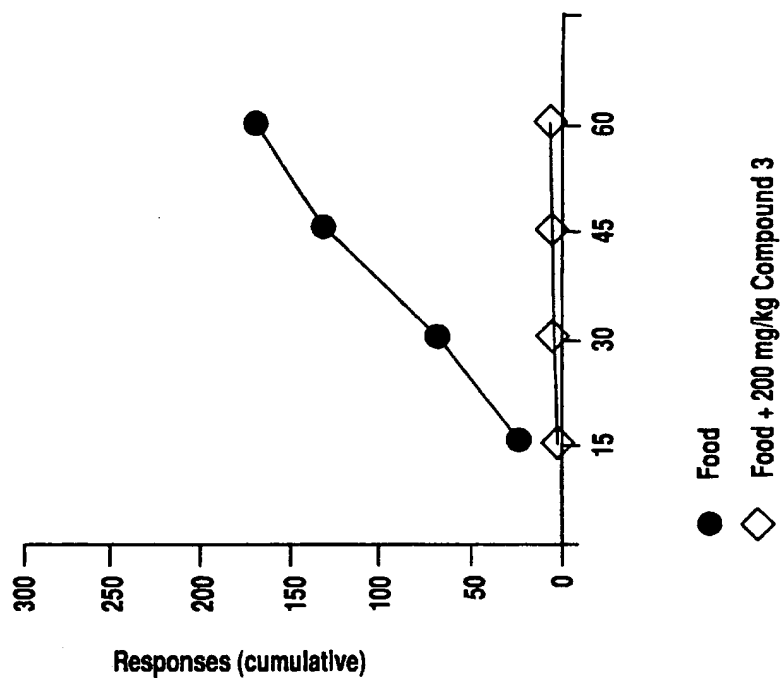
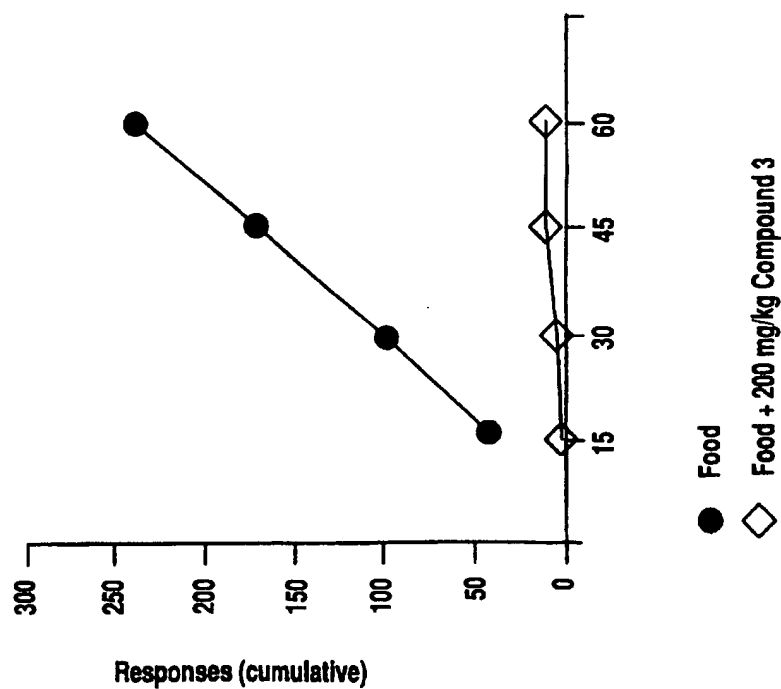


FIG.17A

LE11



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14417

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/44
US CL : 514/574, 921

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/574, 921

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---	US 5,500,420 A (MAIESE) 19 March 1996, see the abstract.	1-2 -----
A		3-83
Y	STAUCH et al. NAALADase: A Potential Regulator of Synaptic Glutamate. Zeneca Pharmaceuticals Group. 1994, pages 38-40, especially page 38.	1-83
Y	VORNOV, J. J. Toxic NMDA-Receptor Activation Occurs During Recovery in a Tissue Culture Model of Ischemia. Journal of Neurochemistry. October 1995, Vol. 65, No. 4, pages 1681-1691, especially pages 1681 and 1687.	1-83



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *B* earlier document published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A

document member of the same patent family

Date of the actual completion of the international search

29 SEPTEMBER 1997

Date of mailing of the international search report

30 OCT 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

KEITH MACMILLAN

Telephone No. (703) 308-1235

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.